The importance of Potato mop-top virus (PMTV) in Scottish seed potatoes

Triona Davey B.Sc (Hons) M.Sc

PhD Thesis

Heriot Watt University
&
Science and Advice for Scottish Agriculture

December 2009

Abstract

The key aim of this research was to determine the extent of PMTV infection in Scottish seed potatoes and the critical factors which influence infection. The research incorporated a survey of PMTV infection in susceptible cultivars in Scotland, a glasshouse trial to determine the role of temperature in the transmission of PMTV from the soil to the host plant, and field trials which studied the transmission rate from seed to daughter tubers and the relative contribution of seed and soil inoculum to disease development.

The survey of Scottish seed crops showed that PMTV occurs in all regions of Scotland but is not particularly prevalent even on known susceptible cultivars. The incidence of crops infected by PMTV differed greatly amongst the regions, with more crops grown in Central Scotland being infected than elsewhere. Although the occurrence of PMTV is linked to the powdery scab organism, there is no correlation between the occurrence of powdery scab and PMTV infection. Temperature was found to be an important factor in the occurrence of symptoms of PMTV infection. The incidence of PMTV infection in tubers was similar at 12°C and 19°C but spraing was absent at 19°C. Transmission from seed to daughter tubers was found to be inefficient, with less than half the daughter tubers derived from PMTV-infected seed being infected by PMTV. However, high incidences of tuber infection were often present in crops after one growing season indicating that soil inoculum is the main source of PMTV infection. Seed-borne inoculum is also of great importance as planting infected seed tubers in clean land brings a risk of introducing PMTV into the soil.

Dedicated to Rudi Boa

Acknowledgements

My warmest thanks go to my primary supervisors Gerry Saddler and Stuart Carnegie at SASA. Their supervision and guidance has been invaluable. Furthermore, their consistent support has been key to my enjoyment of the entire research process. I would also like to thank Wilf Mitchell at Heriot-Watt University for the time taken to read drafts of the thesis and for the support along the way.

I also wish to thank my friends and colleagues, many of whom have acted as sounding boards for ideas and theories as my research progressed. I would specifically like to thank Adrian Fox and Ross Holmes in the Virology Section at SASA for all of their help with the testing and for keeping my spirits up when faced with a mountain of tubers. I couldn't have done it all without you! I would also like to thank Isla Browning for her help and advice. Many thanks also go to the Potato Section, for all of the help throughout the years. My sincere thanks also go to everyone at DMB, in particular Fiona, Vince, Ellen, Craig, Alex and Robert. Thanks for putting up with me! These thanks extend also to the whole of SASA for the opportunities and supportive environment within which to work.

Since I began the PhD process my family have consistently and unconditionally supported and encouraged me in so many different ways. I am eternally grateful.

Finally, I want to thank Rob, who has put up with my moods, my stress, my lack of free time and everything else that goes with the PhD process.

Contents

List of Tables

List of Figures

List of abbreviations

AMP	Amprica Multi Plastics
ANOVA	Analysis of variance
BCE	Before the Common Era
BPC	British Potato Council
BNYVV	*Beet necrotic yellow vein virus*
BSBMV	*Beet soil-borne mosaic virus*
Ct	Cycle threshold
Cv.	Cultivar
Cvs	Cultivars
DARDNI	Department of Agriculture and Rural Development Northern Ireland
DAS-ELISA	Double antibody sandwich enzyme-linked immunosorbant assay
d.f.	Degrees of freedom
DNA	Deoxyribonucleic Acid
EC	European Community
EEC	European Economic Community
ELISA	Enzyme-linked immunosorbant assay
EPPO	European and Mediterranean Plant Protection Organisation
EU	European Union
FAM	6-carboxy-fluorescein
FAO	Food and Agriculture Organisation of the United Nations
FERA	The Food and Environment Research Agency
GIS	Geographical Information Systems
GPS	Geographical Positioning System
HMSO	Her Majesty's Stationary Office
ICTV	International Committee on Taxonomy of Viruses
IPM	Irish Potato Marketing Ltd.
LiCl	Lithium Chloride
LSD	Least Significant Difference
M-MLV	*Moloney murine leukaemia virus*
NTC	No template control
OECD	Organisation for Economic Co-operation and Development
PBS	Phosphate Buffered Saline
PBST	Phosphate Buffered Saline Tween

PCR	Polymerase Chain Reaction
PLRV	*Potato leaf roll virus*
PMTV	*Potato mop-top virus*
PVY	*Potato virus Y*
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
SASA	Science and Advice for Scottish Agriculture
SBCMV	Soil-borne cereal mosaic virus
SCRI	Scottish Crop Research Institute
SE	Super Elite
SGRPID	Scottish Government Rural Payments and Inspections Directorate
SBWMV	*Soil-borne wheat mosaic virus*
SOLA	Specific Off-Label Approval
SSPCSS	Scottish Seed Potato Classification Scheme
TAMRA	Tetramethyl-6-Carboxyrhodamine
TC	Tissue culture
TGB	Triple Gene Block
TRV	*Tobacco rattle virus*
UK	United Kingdom
USA	United States of America
WYSV	*Watercress yellow spot virus*

Publications linked to this work

Carnegie, S. F., **T. Davey** and G. S. Saddler (2009). Effect of temperature on the transmission of *Potato mop-top* virus from seed tuber and by its vector, *Spongospora subterranean*. *Plant Pathology*, in press. Doi: 10.1111/j.1365-3059.2009.02187.x.

Davey T. (2008). An accelerated soil bait assay for the detection *of Potato mop-top Virus* (PMTV) in agricultural soil. *Plant Pathology Techniques and Protocols by Robert Burns (Editor). Humana Press.*

Davey T., I. Browning, S. F. Carnegie, W. J. Mitchell & G. S. Saddler (2008). Soil: The Principal Source of Potato Mop Top Virus (PMTV). Proceedings Crop Protection in Northern Britain 2008. pp. 205-210.

Davey T., I. Browning, S. F. Carnegie & G. S. Saddler (2007). Soil: The principal source of PMTV infection. The 13th European Association for Potato Research Virology Section Meeting.

Davey T., I. Browning, S. F. Carnegie & G. S. Saddler (2007). The importance of potato mop top virus (PMTV) in Scottish seed potatoes. www.potato.org.uk. (British Potato Council) Project no. R247.

Davey T., I. Browning, S. F. Carnegie & G. S. Saddler (2006). The importance of potato mop top virus (PMTV) in Scottish seed potatoes. Proceedings Crop Protection in Northern Britain 2006. pp. 375-380.

Davey T., I. Browning, S. F. Carnegie & G. S. Saddler (2006). The epidemiology of PMTV in seed potatoes. 8th Conference of the European Foundation for Plant Pathology.

Oral Presentations:

Association of Applied Biologist's Meeting: Advances in plant virology (Warwick, 2006).

8th Conference of the European Foundation for Plant Pathology & BSPP Presidential Meeting 2006 (Copenhagen, 2006).

13th European Association for Potato Research. Virology Section Meeting (Aviemore, 2007).

Crop Protection in Northern Britain 2008 (Dundee, 2008).

Thesis overview

This thesis has been separated into five chapters. The first chapter provides a comprehensive overview of the literature relating to *Potato mop-top virus*, while the subsequent three chapters report on the three distinct parts of the work carried out. Chapters 2, 3 and 4 are presented as self contained units, with each consisting of an introduction, methodology, results and discussion section.

Chapter two presents the findings of a survey of four PMTV susceptible cultivars commonly grown in Scotland, which was carried out to determine the incidence of PMTV infection and symptoms of infection.

Chapter three outlines findings from field trials and extensive direct tuber testing to study the transmission of PMTV from infected seed tubers to daughter tubers.

Chapter four presents the findings from a survey focusing on one potato cultivar, cv. Cara. The survey studies PMTV transmission through infected seed tubers and soil infested with viruliferous *Spongospora subterranea* to determine which of these sources of inoculum is the most important in causing economic outbreaks of PMTV infection.

The conclusions from the work presented in Chapters 2 - 4 are presented in Chapter 5.

Chapter 1. Introduction

1.1 The Potato

1.1.1 Taxonomy

The potato (*Solanum tuberosum*) is a member of the family *Solanaceae*; one of the largest and most diverse plant families with over 3000 species (Knapp *et al*., 2004), many of which evolved in South America. Many species in the *Solanaceae* family are used for both food and medicinal purposes (Knapp *et al*., 2004), despite also being known for their toxic properties (Müller, 1998); consequently, they are also commonly referred to as the nightshade family (Korpan *et al*., 2004; Müller, 1998). The leaves, stems and shoots of the potato plant contain high concentrations of the glycoalkaloids α-solanine and α-chaconine (Ji *et al*., 2008; Langkilde *et al*., 2008); produced as part of the plant's natural defences against disease and natural predators (Korpan *et al*., 2004). If large amounts are ingested by humans, the effects can be fatal (Korpan *et al*., 2004; Langkilde *et al*., 2008); however, in smaller doses, these glycoalkaloids have previously been used to treat epilepsy and recent research has highlighted other potential chemotherapeutic properties (Ji *et al*., 2008; Langkilde *et al*., 2008).

Potato shares the genus *Solanum*, the largest genus in the *Solanaceae* family, with over 1000 species (OECD, 2008) including tomato (*Solanum lycopersicum* syn. *Lycopersicon esculentum*) and aubergine (*Solanum melongena*) (Knapp *et al*., 2004). *S. tuberosum* is divided into two subspecies; subsp. *tuberosum* and subsp. *andigena* (Hosaka *et al*., 1988; Huaman *et al*., 2002). *S. tuberosum* subsp. *andigena* is specifically suited to cultivation at high altitudes and short daylight hours whereas subsp. *tuberosum* prefers cultivation at lower altitudes and a longer day length (OECD, 2008).

1.1.2 The History of the Potato

There are almost 200 species of wild potato, all of which are restricted to the Americas (Hijmans et al., 2001). Peru has the largest number of wild potato species (47% of the total) and also the largest number of rare species (Hijmans et al., 2001). The earliest evidence of the use of potato (S. tuberosum subsp. andigena) as a food source dates back to 6000 Before the Common Era (BCE), when early settlers in the Peruvian Andes

began cultivating wild potato species (Salaman et al., 1985). Ugent et al. (1982) also described a collection of 21 preserved tubers dating back to 2000 BCE gathered from 4 archaeological sites in Peru, indicating the persistence of the potato in the diet of early Peruvians.

Salaman *et al*. (1985) described archaeological evidence of potato use from 200 AD (Proto-Chimú and Proto-Nazca periods) through to the Chimú (800-1100 AD) and Inca periods (1100-early 1500s). Specifically, images of potatoes were found to be sculpted onto pottery, indicating the importance of the potato in Peruvian society and culture. As Glave (2001) explained, the development of ancient Andean civilisations was profoundly influenced by the potato; Jesuit Bernabé Cobo, in 1653, even went so far as to term the potato 'the bread of the Indians'. Glave (2001) also noted that the high level of social development attained by the Andean peoples was, in fact, based on the energy provided by their staple food – the potato. This is not surprising considering the nutritional value of potatoes which are rich in Vitamin B, C, iron, magnesium and potassium (FAO, 2008a, Potato Council Ltd, 2008, True *et al*., 1978).

The potato appears to have arrived in Europe during the early 16th Century with Francisco Pizarro's return to Spain following his conquest of the Inca Empire (Harding, 1993). Initially, Europeans were sceptical about potato as a food source due to a variety of superstitions (Reader, 2008) and they were cultivated by farmers primarily as feed for livestock for some time, before gradually becoming an important part of the human diet (Chapman, 2000). The original potatoes to arrive from South America appear to have resembled *S. tuberosum* subsp. *andigena*; subsequent artificial selection of seedlings under long daylight conditions gradually led to the development of the long-day adapted type *S. tuberosum* subsp. *tuberosum* (Burton, 1989; Hosaka *et al*., 1988).

From Spain, the potato quickly spread throughout the rest of Europe, most probably by sea, with sailors frequently bringing a supply of tubers on board to prevent scurvy (www.potatoes.com/Growing-History). By 1600, the potato had been introduced into Italy, Austria, Belgium, Holland, France, Switzerland, England, Germany and Portugal (Brown, 1993; Chapman, 2000; Hougas, 1956). By 1620, the potato had arrived in North America (Laufer and Martin, 1938; Ochoa, 2001) as a British export and appears to have arrived in Ireland a few years later in 1625 (Ochoa, 2001). By the 1850's, the potato was already well established in Scotland, Norway, Sweden and Denmark and by

the end of that century was being cultivated in nearly all of Eastern Europe (Ochoa, 2001).

Ireland proved to be a particularly eager consumer, with the Irish diet often depending almost entirely on potatoes during the period 1700 to the mid-1850s (Connell, 2002). This dependence was due to a number of factors including low costs, availability and the suitability of the Irish climate and soil type (Burton, 1989; Kinealy, 1997). However, the consequences of this limited diet proved disastrous when the potato crop in Europe was affected by late blight (*Phytophthora infestans*), which originated in Mexico in 1840 (Andrivon, 1996; Mulder and Turkensteen, 2005). Ireland was badly affected with the population halving between 1845-1849 (The Great Famine) because of death from starvation and mass emigration (Kinealy, 1997).

Potato remains an important food source today and is grown on every continent, in over 130 countries and in almost all climatic conditions (Wale *et al*., 2008). It is the World's fourth largest food crop, exceeded only by maize, wheat and rice (Stevenson *et al*., 2001); no other food can match the potato for production of food energy and value per unit land area (Mohammadi *et al*., 2008). The Food and Agriculture Organisation of the United Nations (FAO) estimated that international trade in potatoes was worth $US 6 billion in 2008 (FAO, 2008b). In recent years, potato production has become increasingly important in developing countries; however, this has been paralleled with static production in developed countries (Figure 1-1). Since 2003, China has become the World's single biggest producer of potatoes (Figure 1-2).

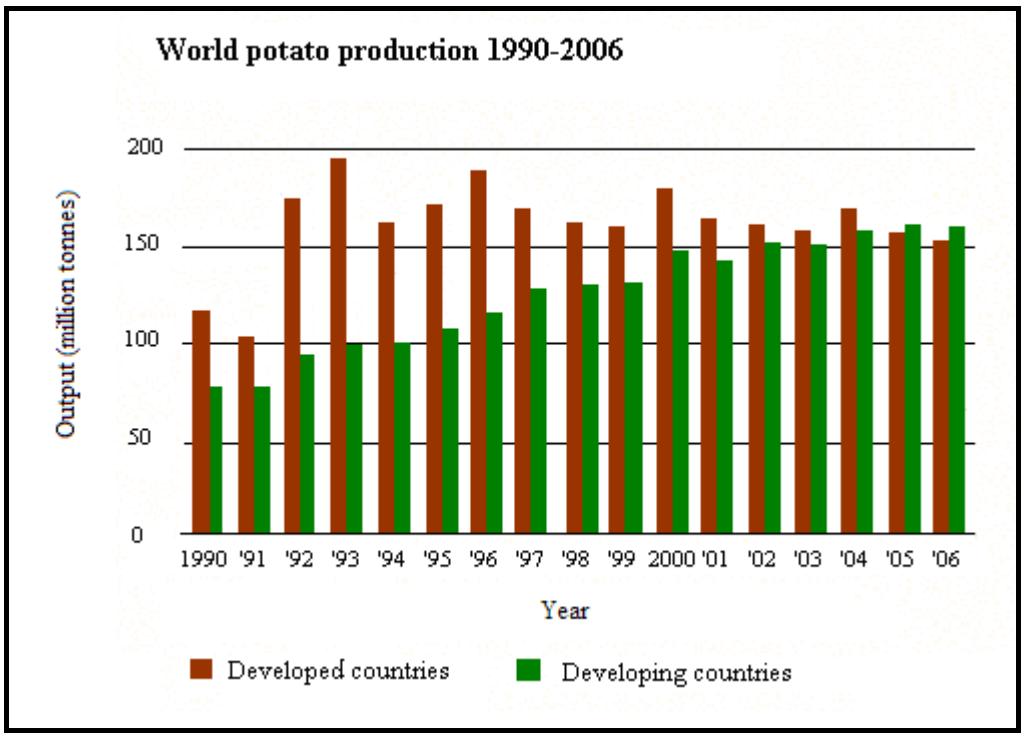

Figure 1-1. Trend in world potato production from 1990-2006 in both developing and developed countries (FAO, 2008b)**.**

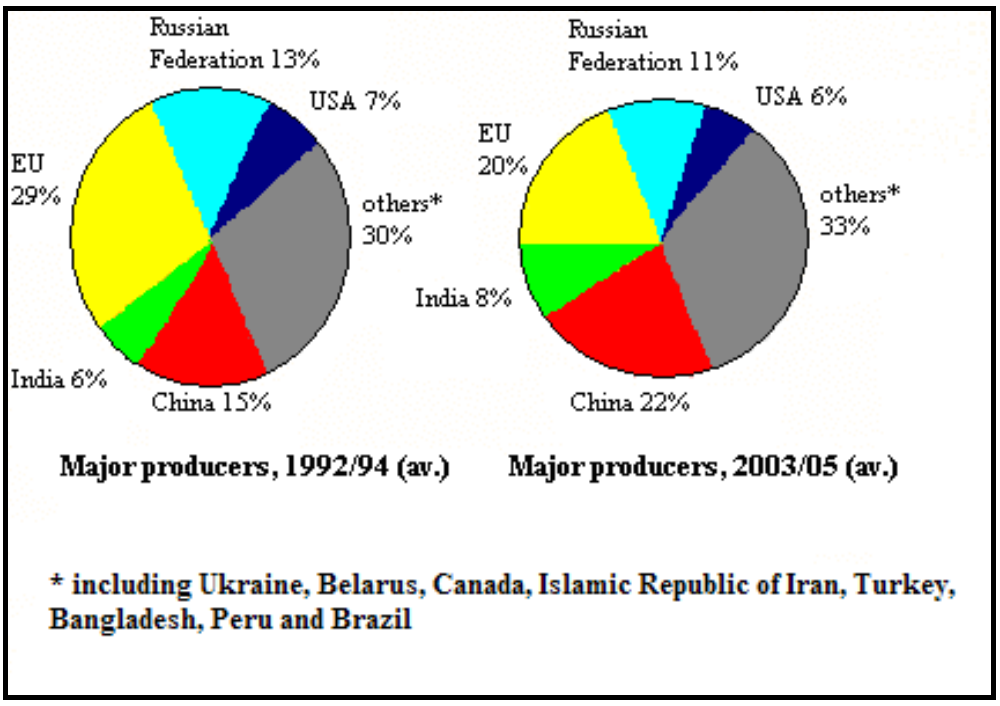

Figure 1-2. Major producers of potatoes worldwide from 1992-1994 and 2003-2005 (FAO, 2008b).

1.1.3 Propagation

Potato, an annual, herbaceous dicotyledonous plant, is primarily propagated vegetatively from seed tubers. The seed tubers are generally 35-55mm in size at the time of planting. Figure 1-3 illustrates the six main stages of potato multiplication:

- 1) The sprouted seed tuber at time of planting
- 2) Tuber sprouting
- 3) Plant emergence and shoot expansion (stolon initiation usually occurs close to or shortly after the time of plant emergence)
- 4) Plant emergence and shoot expansion
- 5) Tuber initiation
- 6) Tuber bulking.

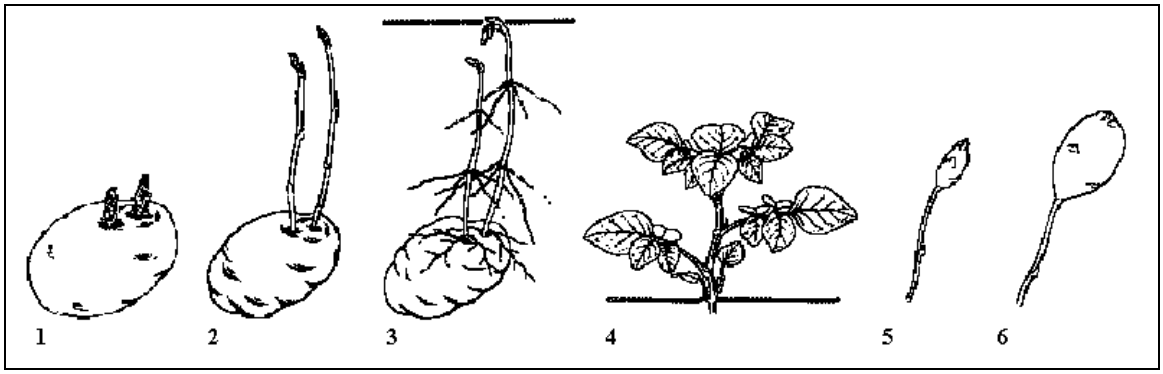

Figure 1-3. The six main stages of potato multiplication (adapted from Jeffries and Lawson, 1991).

Maintenance of varietal distinctness and genetic purity is a significant advantage of vegetative propagation (Zimmerer, 2002); however, it can also lead to gradual deterioration of seed quality with each multiplication because of the progressive accumulation of virual, fungal and bacterial pathogens (Kaur and Mukerji, 2004).

1.2 Potato Production and Seed Classification in Scotland

The potato industry is an important contributor to the Scottish economy with the total value of the 2008 crop exceeding £190 million (Scottish Government, 2009a). Potato production in 2008 was 7,800 tonnes (400 hectares) early ware[1], 841,100 tonnes (18,600 hectares) ware and 428,500 tonnes (10,800 hectares) seed (Scottish Government, 2009a). In 2008, 65,000 tonnes of Scottish seed potatoes were exported to countries outside the EU (Scottish Government, 2008). Scotland has a long history of seed potato production and variety development. The country's cool climate has discouraged aphid survival and flight and resulted in seed that is relatively free from tuber-borne virus (Rennie, 2001). In total, approximately 80% of the United Kingdom's seed potatoes are produced in Scotland (DARDNI, 2007; FERA, 2008; SASA, 2007), indicating the importance of Scottish seed potatoes to both the Scottish economy and the UK's potato industry.

Seed potatoes produced and marketed in Scotland are required to meet the health and varietal purity requirements set out in The Seed Potatoes (Scotland) Regulations (Scottish Government, 2000). These enact in national legislation the EC Council Directives 93/17/EEC (European Union, 1993) and 2002/56/EC (European Union, 2002), which are concerned with the marketing of seed potatoes in the EU. SASA (Science and Advice for Scottish Agriculture), part of SGRIPD (Scottish Government Rural Payments and Inspections Directorate), operates the Scottish Seed Potato Classification Scheme (SSPCS) and is the Certifying Authority for seed potatoes in Scotland. Nearly all seed potato crops grown in Scotland originate from *in vitro* microplants (Nuclear Stock) produced by SASA; these microplants are extensively tested for a number of indigenous and non-indigenous pathogens and checked for off-types/variants (Figure 1-4). This official control assures a very high level of plant health at the start of the propagation chain. Scotland is recognised as an EU 'Community Grade Region', producing only basic and pre-basic seed potatoes. To protect crops from external sources of virus, the seed potatoes planted to produce ware crops must be either classified or once-grown farm saved seed.

[1] Eating potatoes are referred to as ware potatoes; early ware potatoes are the first potatoes to be harvested (generally 60-90 days after planting).

Figure 1-4. Various stages of propagation of microplants. Microplants are multiplied up in the laboratory by sub-dividing them into nodal cuttings, each of which grows into a new microplant. Subdivision continues until the numbers required for transplanting are produced.

Nuclear stock microplants are distributed to a small number of approved growers for further multiplication, mostly in a protected environment, to produce mini-tubers (class Pre-basic TC seed potatoes). Mini-tuber production takes place in a range of growing media, peat or hydroponics, resulting in the production of disease free mini-tubers. These initial tubers are then multiplied in the field by approved Pre-basic growers who are required to maintain the highest standards of crop husbandry to limit the spread of pests and disease, e.g. extended crop rotations, and the use of separate stores for Pre-basic crops. The production of Pre-basic seed potatoes is limited to 4 field generations after which they can be entered for classification as basic category seed potatoes. Figure 1-5 shows the classification pathway for the SSPCS. The number of years for which stocks can be entered for classification is limited and each class is designated with a number which is the number of years for which the stock has been classified at that class. In theory, seed can be multiplied in the field for 10 years; however, most multiplication programmes only run for 4-6 years.

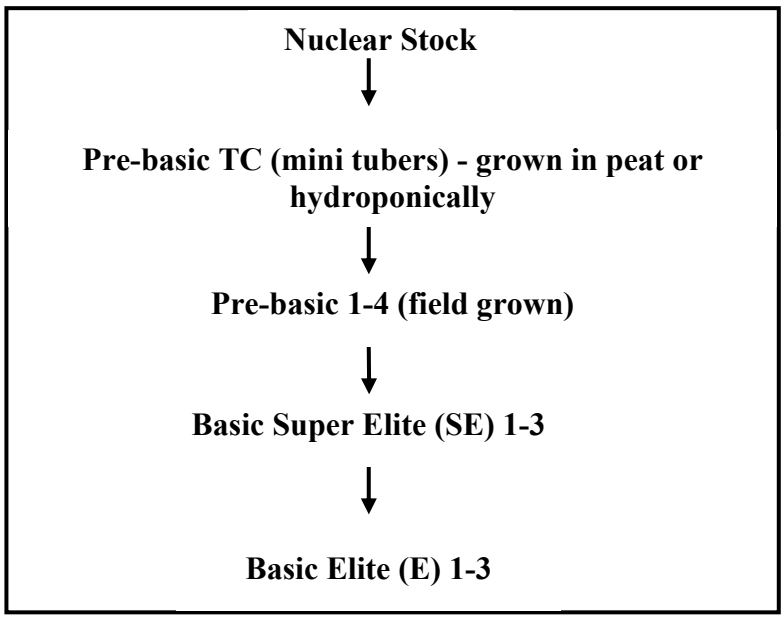

Figure 1-5. Classification pathway and classes of Scottish seed potatoes. Numbers (1-3 or 1-4) indicate the number of years the stock has been classified at each class (Rennie, 2001).

Seed crops are officially inspected twice during the growing season and assessed against strict tolerances for varietal purity, aphid numbers, virus diseases and blackleg (Table 1-1). Prior to planting a seed potato crop, the land must be confirmed free of wart disease and found free of potato cyst nematode. The minimum interval between potato crops is 7 years for Pre-basic crops and 5 years for Basic crops. After seed potatoes are prepared for harvesting, a sample of tubers is inspected for rots, blemishes and defects. The inspector must ensure that tolerances are met prior to the seed being marketed.

Table 1-1. Disease and varietal purity tolerances applied at growing crop inspection of seed potato crops of various classes in Scotland (Scottish Government, 2008a).

	Maximum % plants affected at growing crop inspection			
Seed class/ Faults	**Pre- basic**	**SE**	**E**	**A**
Leafroll virus	0	0.01	All virus 0.5 including 0.1 severe virus	All virus 1.0
Severe mosaic	0	0		
Mild mosaic	0	0.05		
Blackleg	0	0.25	0.5	1.0
Deviations from variety and type	0	0.05	0.05	0.1

1.3 Diseases of Potato

Potato is host to a wide variety of pathogenic organisms, including bacteria, fungi, nematodes, viroids and viruses. Losses can occur when crops are growing, at lifting and during storage of harvested tubers. Not all diseases result in the destruction of tubers; however, disease symptoms such as surface blemishes can often cause a significant decrease in marketable value (Kaur and Mukerji, 2004). Strategies for disease control rely on preventive measures to regulate trade and cultivation, the use of resistant varieties, control of vectors, chemical control, and the detection and elimination of contaminated plants and seed.

There are more than 40 viruses that affect potato (Valkonen, 2007). Potato viruses are of major economic importance to the UK potato industry with an approximate loss of over £13 million in 2002 due to virus infection (Andret-Link and Fuchs, 2005). Virus infection in a potato crop can result in a wide variety of symptoms developing on infected plants causing loss of yield and quality. Infection by two or more viruses invariably results in severe symptoms and a significant loss of yield. Latent infections can cause significant problems as affected plants provide a source of inoculum for other plants. The consequences can be serious if virus is transmitted to susceptible varieties.

The majority of plant viruses and viroids rely on a vector for transmission amongst plants (Singh *et al*., 2008). There are three main routes of transmission for potato viruses: aphids (e.g. *Potato virus Y* (PVY) and *Potato leaf roll virus* (PLRV)) (Radcliffe and Lagnaoui, 2007), soil-borne (e.g. *Tobacco rattle virus* (TRV) and *Potato mop-top virus* (PMTV)) (Calvert, 1968); and tuber-borne (e.g. Potato spindle tuber viroid (PSTVd)) (de Hoop *et al*., 2008). Aphids represent the main route of transmission for plant viruses, being responsible for spreading just over half (55%) of the 550 vector-transmitted viruses; 7% are nematode transmitted and 5% transmitted by fungi and plasmodiophorids, with the remaining 33% spread by various arthropod vectors (Andret-Link and Fuchs, 2005).

Over 50 viruses belonging to 12 genera have been identified as soil-borne viruses (Verchot-Lubicz, 2003). Generally, soil-borne viruses do not remain in the soil as 'free' virus and are vectored by fungi, fungus-like organisms and nematodes (Singh *et al*., 2008; Verchot-Lubicz, 2003). These viruses represent major production constraints and can persist in field soil for many years (Agrios, 2005). Fungi and fungus-like organisms represent a significant group of vectors for these viruses with 5 species responsible for the transmission of 30 soil-borne viruses or virus-like agents (Singh *et al*., 2008). Less than 1% of all plant-parasitic nematodes transmit viruses; however, nematode-transmitted viruses are of global economic importance, primarily for fruit and vegetable crops (Verchot-Lubicz, 2003). Although direct feeding by nematodes can cause severe damage to the host, some species (*Trichodorus* and *Paratrichodorus* spp.) are also important due to their ability to transmit tobraviruses (Holeva *et a*l., 2006; Taylor and Robertson, 1970). *Trichodorus* and *Paratrichodorus* spp. are generally found in light sandy soils and have been reported in Europe, N. America, Japan and China (Dale and Neilson, 2006). Mixed populations of nematodes can have a detrimental effect on plant growth (due to root damage) (Holeva *et al*., 2006), yield and tuber quality (Dale and Neilson, 2006). Of the three tobraviruses transmitted by free-living nematodes, only one, TRV, affects potato. Infection by TRV causes spraing symptoms (necrotic arcs or flecks in the flesh of tubers) in tubers which severely reduce their marketability (Agrios, 2005) and is of economic importance in potato growing regions of North America and Europe (Cooper and Harrison, 1973).

Fungi and fungus-like organisms known to transmit viruses belong to the orders *Chytridales* and *Plasmodiophorales.* There are 3 known genera in the order *Plasmodiophorales: Olpidium*, *Spongospora* and *Polymyxa* (Verchot-Lubicz *et al*., 2007), all of which are zoosporic parasites of plant roots (Verchot-Lubicz, 2003). Three species in the Order *Chytridales* transmit plant viruses: *Olpidium brassicae*, *O. radicale* (Adams, 1991) and *O. cucurbitacearum* (Verchot-Lubicz, 2003). Many viruses transmitted by the *Olpidium spp.* cause necrotic diseases of agronomic importance e.g. *lettuce big-vein* and *tobacco necrosis viruses* (Campbell and Fry, 1966).

The taxonomic status of the *Plasmodiophorales* is problematic and in the past they have been classified with protozoans (De Bary, 1884), and also oomycete fungi (Karling, 1968). Their taxonomic status remains open to debate; however, the informal term "Plasmodiophorids" is generally used (Merz *et al*., 2005). All plasmodiophorids are obligate parasites of higher plants and multiply intracellularly (Ward and Adams, 1998). The plasmodiophorids have a zoosporangial stage in their life cycle (see Figure 1-6) (Kole and Gielink, 1963) which occurs after germination of resting spores (Dessens and Meyer, 1996). A number of plasmodiophorids represent important plant pathogens and the majority of what are known as 'fungal' virus vectors are included in this group (Merz *et al*., 2005). Three species of plasmodiophorid act as viral vectors: *Polymyxa betae*, *P. graminis* (Ward and Adams, 1998) and *Spongospora subterranea* (Clay and Walsh, 1997), transmitting approximately 20 plant viruses between them (Adams *et al*., 2001). *P. betae* is the vector for *Beet necrotic yellow vein virus* (BNYVV), the causal agent of sugarbeet rhizomania (Verchot-Lubicz *et al*., 2007). *P. graminis* is the vector for a number of viruses which occur on grains and grasses (Ward and Adams, 1998).

There are three species of the genus *Spongospora: S. subterranea* (f. sp. *subterranea and* f.sp. *nasturtii), S. campanulae* and *S. cotulae* (Karling, 1968). Two members of the genus are plant pathogens as well as 'fungal' virus vectors: *S. subterranea* f. sp. *subterranea* and *Spongospora subterranea* f.sp. *nasturtii* (Merz *et al*., 2005). *S. subterranea* f.sp. *nasturtii* is the causal agent of crook rot disease in watercress. The pathogen infects the roots of plants causing distortion and stunting of the roots and chlorosis in the above ground parts of the plant (Down *et al*., 2002). *S. subterranea* f.sp. *nasturtii* is also the vector for *Watercress yellow spot virus* (WYSV), which is of major economic importance in France and England (Arnold *et al*., 1995; Walsh *et al*.,

1988). *S. subterranea* f. sp. *subterranea* causes the disease powdery scab on potatoes and acts as the vector for PMTV.

1.3.1 *S. subterranea* and Powdery Scab

Powdery scab is an increasingly important disease of potatoes which occurs in potato growing areas worldwide. The disease was first reported in Germany in 1842 when Wallroth described the symptoms (Merz, 2008; Termorshuizen, 2007) and was subsequently recorded in the UK at the end of the 1840s (HMSO 1981). The distribution of powdery scab appears to be increasing and in the past two decades the disease has been reported for the first time in Pakistan (Ahmad *et al*., 1996), Alaska (Carling, 1996), North Dakota (Draper *et al*., 1997), Costa Rica (Montero-Astúa *et al*., 2002), Korea (Kim *et al*., 2003) and Malta (Porta-Puglia and Mifsud, 2006). Nakayama *et al*. (2007) found powdery scab in almost 10% of the total potato production area of Hokkaido, the most northern island of Japan. Powdery scab is also a persistent problem in Scotland, particularly for seed growers in the north and north-east of the country (Wale, 2000).

The spread and increasing importance of powdery scab worldwide may be due to a number of factors including: the popularity of cultivars that are particularly susceptible, the increased use of irrigation that can result in favourable conditions for disease development, inadequate crop rotations and the durability of long lived soil inoculum (Qu, *et al.*, 2006; Wale, 2000; de Haan and van den Bovenkamp, 2005; Harrison *et al.*, 1997). Powdery scab usually only causes cosmetic damage to tubers, making them appear unsightly and affecting their marketability (Wale, 2000). It is likely that the increasing importance of the disease is the result of changing consumer preferences. Consumers today prefer washed pre-packed potatoes and a clean, blemish free appearance is, therefore, a critical marketing attribute.

In order to restrict the introduction and spread of the disease, many importing countries have set strict tolerances for powdery scab symptoms on seed tubers. As a result, seed lots for export may be rejected at inspection if powdery scab is present, even at low levels (De Haan and van den Bovenkamp, 2005); this in turn may have a detrimental effect on export opportunities and may also impose a financial burden on the grower. It is estimated that the combined economic losses resulting from both a reduction in marketable yield and the increased cost of disease control can be as high as £7 million

per annum in Scotland alone (Wale, 2003). The Scottish minimum standards for powdery scab on seed potatoes being marketed within and out with the EU are shown in Table 1-2, along with a number of examples of countries that have set stricter tolerance levels.

Table 1-2. Tolerances set for powdery scab in the Scottish Seed Potato Classification Scheme and importing countries with stricter tolerance levels (Scottish Government 2008b).

Minimum Export Standards	**Tolerance by weight**	**Allowable % surface area**
UK Basic Seed (within EU)	3%	12.5%
Minimum export tolerance for Basic Seed (outside EU)	1.5%	12.5%
Falkland Islands	0.5% Basic 1.5% Pre-Basic	n/a
Kuwait	About 50% of the number of tubers is free from scab, whereas the remaining 50% may have 1 to 2 small shallow lesions covering max. 1/32 of the tuber's surface.	
Pakistan	As for Kuwait	
Thailand	No more than 1.5% of tubers by weight should have more that 5% surface area cover	
Cuba	0.1%	12.5-25%
Iran	0%	0%
Iraq	0%	0%
Egypt	0%	0%
Brazil	0%	0%
Cuba	0%	0%
Morocco	0%	0%
Jamaica	0%	0%
Jordan	0%	0%

1.3.1.1 Life cycle of S. subterranea and factors affecting disease development

S. subterranea can survive in the soil for at least 15 years (Calvert 1968) as thick-walled resting spores commonly found in aggregate form (spore balls or cystosori), each containing 100 or more spores (Harrison *et al*., 1997). Under wet conditions, the resting spores germinate and release one primary biflagellated zoospore (Bell *et al*., 1999). These primary zoospores can swim short distances and attach to, and penetrate potato root tissue, stolons, young shoots and tubers (Hims and Preece, 1975) (Figure 1-6).

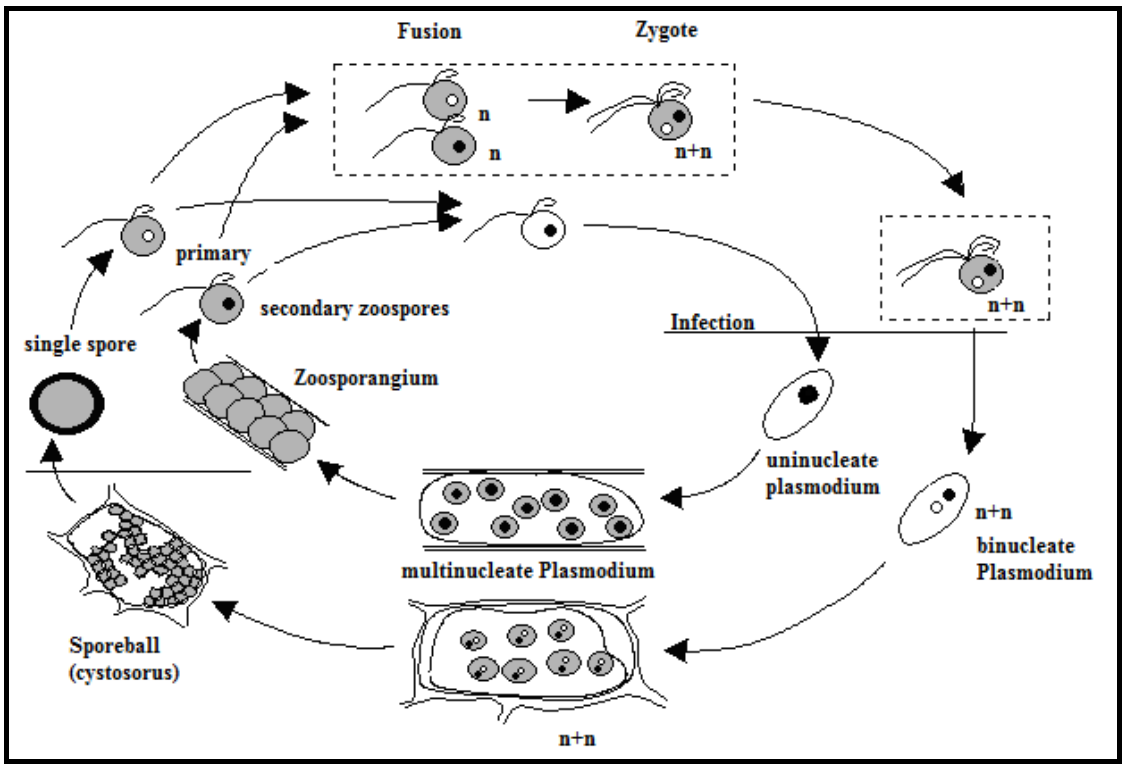

Figure 1-6. Life cycle of the powdery scab pathogen; *S. subterranea* (Merz, 2008).

After penetration, pathogen growth and development continues (Figure 1-6). The zoospore becomes a multinucleate plasmodium, increasing in size and eventually cleaving into segments to form uninucleated zoosporangia. Each of these contains 4 to 8 secondary zoospores that can further infect plant tissue, typically developing tubers and roots (Figure 1-7) (Hims and Preece, 1975). This results in progressively more zoospores entering the soil and inoculum levels increasing, if conditions are suitable (Kole and Gielink, 1963). Infection of the tubers by zoospores occurs most frequently during tuber initiation (normally 5-7 weeks after planting) (Harrison *et al*., 1997). The first symptoms of powdery scab are small, usually pimple-like swellings (Figure 1-8). On mature tubers, powdery scab presents itself as tiny, hollow lesions filled with brown powder consisting of sporeballs (Germundsson *et al*., 2002) (Figure 1-9). Later on, pustules can increase in size and rupture the epidermis (de Haan and Van den Bovenkamp, 2005) (Figure 1-10).

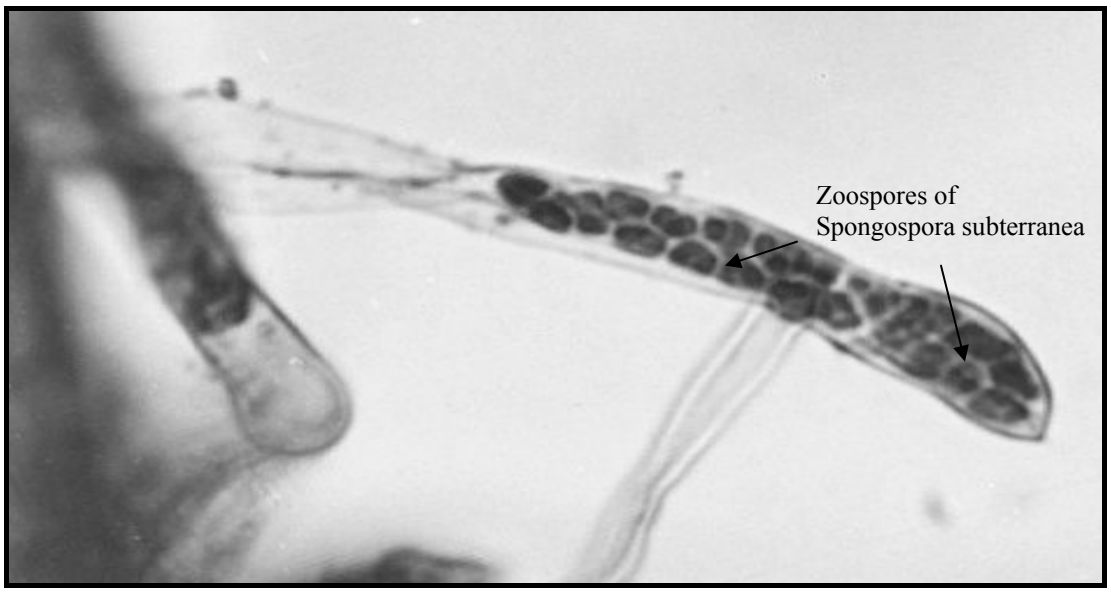

Figure 1-7. *S. subterranea* zoospores in root hair of potato cv. Arran Pilot. (Photograph: Scottish Crop Research Institute).

Figure 1-8. Early symptoms of powdery scab infection – pimple-like swellings on the surface of a tuber of a tuber (cv. Nicola).

Figure 1-9. Powdery scab lesions on a tuber surface (cv. Pentland Squire) caused by *S. subterranea*.

Figure 1-10. An example of severe powdery scab symptoms on a potato tuber of cv. Saturna.

Scabs normally only affect the outer tissue but occasionally they may penetrate more deeply, effectively destroying a large portion of the tuber (Harrison *et al*., 1997). Infection through the lenticels leads to the formation of scabs, whereas infection through the eyes can lead to the less frequent, canker symptoms, which can vary in severity (Wastie *et al*., 1988) (Figure 1-11). Infection of the root or stolon causes abnormal growth of the infected cells and can lead to the formation of root galls (Figure 1-12), containing sporeballs which can be released into the soil thus maintaining or increasing the amount of inoculum in the soil.

Jones and Harrison (1969, 1972) have described a wide host range for *S. subterranea*. They found zoosporangia in plants of the families *Aizoaceae, Boraginaceae, Caryophyllaceae, Chenopodiaceae, Geraniaceae, Papaveraceae, Plantaginaceae, Polygonaceae, Ranunculaceae, Resedaceae, Solanaceae* and *Urticeae*; however, sporeballs were not seen. Qu and Christ (2006) on the other hand recently observed sporeballs in root galls of plants of yellow mustard (*Brassica campestris*), oat (*Avena sativa*) and tomato (*Lycopersicon esculentum*) grown in nutrient solution inoculated with powdery scab scrapings. This indicates that soil inoculum may also be maintained or increased by both solanaceous and non-solanaceous hosts (Qu and Christ, 2006).

Figure 1-11. Canker symptoms tubers of cv. Pentland Squire.

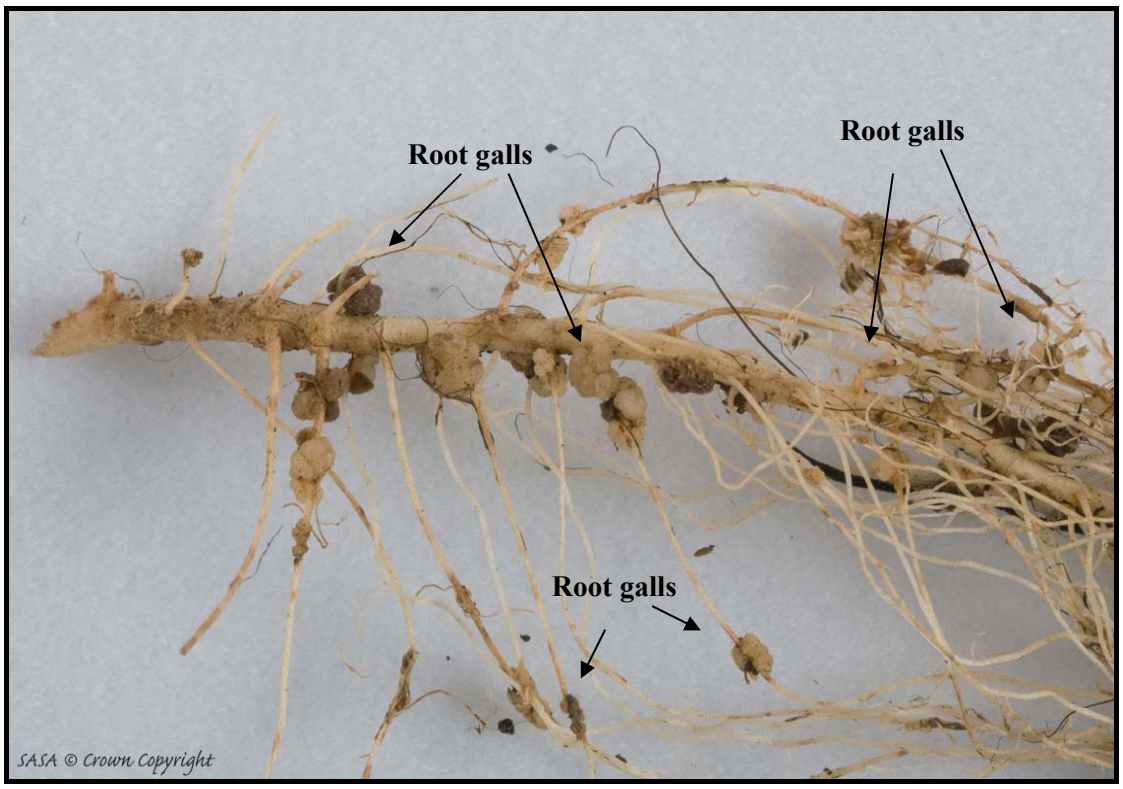

Figure 1-12. Development of root galls on roots and stolons.

Powdery scab is generally associated with early planting in soils that are cool and wet, and with cool and wet growing seasons. The amount of inoculum of *S. subterranea* in soil appears to be relatively unimportant if optimal conditions for disease development occur (van de Graaf *et al*., 2005). Powdery scab can develop at temperatures as low as 9ºC and as high as 17ºC (van de Graaf *et al*., 2002) although it was more prevalent and severe at 12ºC (van de Graaf *et al*., 2005). De Boer *et al.*, (1985) also found that most powdery scab developed at 12.5ºC, with moderate amounts at 15ºC, and low amounts at 17.5ºC and 20ºC. No disease was observed at 10ºC. The optimum temperature for root gall development appears to differ from that for powdery scab. Van de Graaf *et al.* (2002) reported that root galling was more severe at 17ºC than at 12ºC with no root galls at 9ºC.

Soil moisture is also a key factor in the development of powdery scab. Adams *et al.* (1986) illustrated the importance of irrigation on disease development and reported that soil water content during the first half of the growing season had a greater effect on disease development than at other times. De Boer (2000) also reported a positive correlation between soil moisture and incidence of infections as assessed by zoosporangia development in root hairs of cv. Sebago grown in an artificially inoculated

clay soil. The highest incidence of zoosporangia was observed when the soils were near saturation and the incidence decreased proportionally with each reduction in soil moisture content. Wale (2003) and van de Graaf *et al*. (2005) also highlighted the importance of continuous near-saturated conditions for infection to occur. High soil moisture content may facilitate the release of zoospores and enable them to swim greater distances more rapidly in the 'free' soil water, thereby increasing the area of infection. The primary influence of soil type on the occurrence of powdery scab is through the variability in soil water retention (Harrison *et al*., 1997).

1.3.1.2 Detection

Powdery scab on tubers can often be misdiagnosed due to the similarity of the symptoms to common scab (*Streptomyces* spp.) and skin spot (*Polyscytalum pustulans*). At harvest, lesions of common scab resemble those of powdery scab, making visual diagnosis difficult. Skin spot is not normally seen at harvest but develops in store. Symptoms are similar to the first symptoms of powdery scab, small pimple-like swellings. Such similarity in the symptoms of these diseases can result in misdiagnosis. These diagnostic problems can be overcome by various methods. Previously, antibody based methods such as enzyme linked immunosorbant assay (ELISA) were used for the detection of *S. subterranea* on tubers (Harrison *et al*., 1993) and in the roots of bait plants grown in inoculated soil (Merz and Walsh, 1997). However, the specificity and sensitivity of antibody techniques is limited due to the multiple stages in the pathogen's life cycle and the inability of this technique to differentiate between viable and non-viable resting spores. Bulman and Marshall (1998) and Bell *et al*. (1999) developed *S. subterranea* specific primers for use in conventional PCR for the detection of small amounts of the pathogen in soils and on tubers. Both their methods were able to quantify cystosori on tuber samples; however, detection and quantification of the pathogen in soil was inaccurate. The commonly used molecular method of detection is real-time PCR. Van de Graaf *et al*. (2003) and Ward *et al*. (2004) have both developed sensitive and rapid real-time assays for the detection and quantification of *S. subterranea* in soil, water and potato tissue samples. Ward *et al*. (2004) reported that, when comparing dilution endpoints, their real-time PCR assay is 100 times more sensitive than ELISA and conventional PCR; in addition, the real-time assay incorporates an internal potato DNA control and can accommodate differences in efficiency and identify false negatives. However, the assay is intended for routine diagnosis and relies on differences in C_T values to quantify DNA in the sample. Van de

Graaf *et al.* (2003) developed a quantitative assay for detection in soil, water and plant tissue which was 1000 times more sensitive than ELISA. The assay developed by Ward *et al.* (2004) constructed a standard curve using serial dilutions of diseased tissue whereas the quantitative assay developed by van de Graaf *et al.* (2003) constructed a standard curve using a suspension of a known number of sporeballs enabling the number of sporeballs in a sample to be calculated. However, the assay developed by van de Graaf *et al.* (2003) is reliant on a DNA extraction method that is laborious and would not be suitable for high throughput laboratories.

1.3.1.3 Control

Current control measures for powdery scab are limited. Avoidance is the most effective control measure, i.e., by not planting infected seed tubers or by not planting seed in land infested with the pathogen. Control may also be achieved by planting slightly later in the year when the temperature has increased and by reducing irrigation, especially during tuber initiation, when infection is most likely to occur (BPC, 2007). In 2006, a Specific Off Label Approval (SOLA) was granted for the use of the active ingredient Fluazinam® (a pyridinamine) as an in-furrow soil treatment to control the disease in seed crops. In New Zealand, Fluazinam® applied in-furrow at the time of planting powdery scab-free seed tubers reduced the incidence of powdery scab on daughter tubers and significantly increased the marketable yield, irrespective of the rate applied (Falloon *et al*., 1996). Some varieties are more susceptible to powdery scab than others e.g., cvs Estima, Maris Piper and Cara are highly susceptible whereas cvs Saxon, Hermes and Sante are more resistant (BPC, 2007). It appears that no varieties are immune as there are still no known sources of full resistance to powdery scab available.

1.4 Potato Mop-Top Virus (PMTV)

Symptoms of *Potato mop-top virus* (PMTV) were first described in a crop of cv. Kerr's Pink growing in Northern Ireland (Jones and Harrison, 1969); however, it was nearly 10 years later that the virus was identified and formally reported by Calvert and Harrison (1966). Following this first description of PMTV, the virus was subsequently reported in a number of European countries including the Czech Republic (Novak *et al*., 1983), Denmark (Nielsen and Mølgaard, 1997), Finland (Kurppa, 1989b), Ireland (Foxe, 1980), the Netherlands (van Hoof and Rozendaal, 1969), Norway (Bjornstaad, 1969) and Sweden (Germundsson *et al*., 2000). PMTV has also been recorded in Israel (Zimmerman-Gries, 1972), Japan (Imoto *et al*., 1986), Taiwan (ICTV, 2002) and South America (Jones, 1975; Hinostroza and French, 1972; Salazaar and Jones, 1975). More recently, PMTV has been reported as occurring in Canada and the United States (Lambert *et al*., 2003; Xu *et al*., 2004). Todd (1965) recorded the first incidence of PMTV in Scotland with Calvert and Harrison (1966) confirming the presence of PMTV in most commercial stocks in England, albeit at a low incidence. Figure 1-13 shows the worldwide distribution of PMTV.

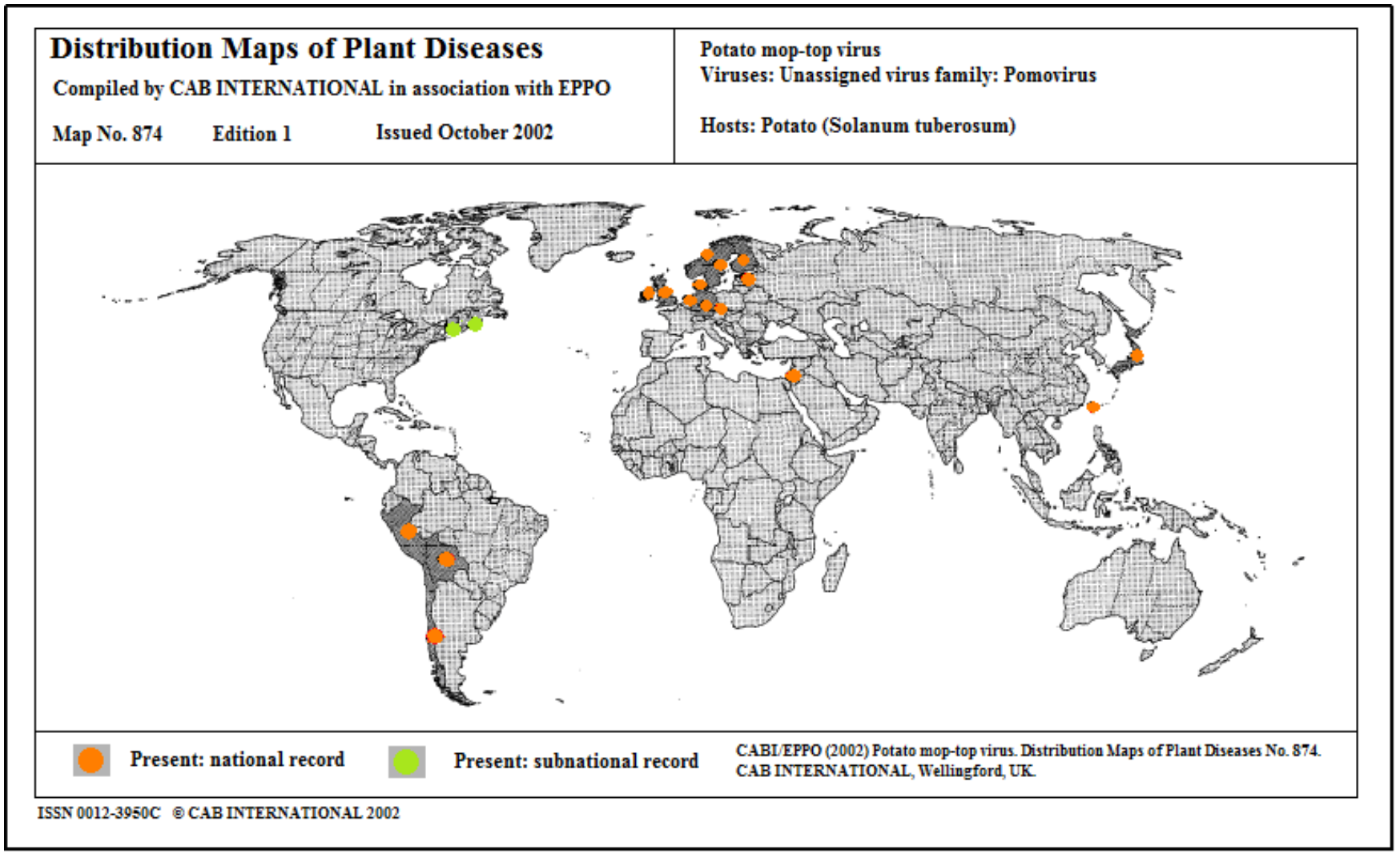

Figure 1-13. Map illustrating the worldwide distribution of PMTV (CABI/EPPO, 2002).

PMTV was originally assigned to the genus Furovirus (Fungal Transmitted rod shaped virus) (Francki *et al*., 1991); however, it has more recently been assigned to the genus *Pomovirus* (Potato mop top virus), primarily on the basis of the number of genome segments (Torrance and Mayo, 1997). PMTV is vectored by the potato powdery scab plasmodiophorid, *S. subterranea* (Arif *et al*., 1995; Calvert and Harrison 1966) (See sections 1.3 and 1.3.1). Arif *et al.* (1995) were able to transmit PMTV to healthy plants using a cystosorus (sporeball) of *S. subterranea* which had previously been virus free but had acquired PMTV from potato plants manually inoculated with a PMTV isolate.

Potato appears to be the most important host for PMTV (Jones and Harrison, 1972); however, in South America, other weed and crop species in the families *Solanaceae*, *Chenopodiaceae* and *Tetragoniaceae* are also known hosts of PMTV (Andersen *et al*., 2002; Brunt *et al*., 1990; Jones, 1981). A number of other plant hosts have been identified by artificial inoculation and are used under laboratory conditions for diagnostics (*Chenopodium amaranticolor*, *Nicotiana debneyi* and *Nicotiana tabacum*) and to maintain the virus (*Nicotiana benthamiana* and *N. debneyi*) (Harrison and Reavy, 2002).

1.4.1 PMTV Genome

PMTV is the type species of the genus *Pomovirus* (Arif and Reavy, 2000). This genus is not assigned to a family. There are four species in the genus *Pomovirus,* including the type species; the others are *Beet soil-borne virus*, *Broad bean necrosis vir*us (Savenkov *et al*., 1999) and *Beet virus Q* (Koenig *et al*., 1998). Pomoviruses are morphologically similar to other rod-shaped viruses, i.e. furoviruses (*Soil-borne cereal mosaic virus* (SBCMV) (Hariri and Meyer, 2007) and *Soil-borne wheat mosaic viru*s (SBWMV) (Shirako and Wilson, 1993)); tobraviruses (*Tobacco rattle virus* (TRV) (Legorburu *et al*., 1996)) and benyviruses (*Beet necrotic yellow vein virus* (BNYVV) (Bouzoubaa *et al*., 1986) and *Beet soil-borne mosaic virus* (BSBMV) (Lee *et al*., 2001)). The main difference between pomoviruses and the other rod-shaped viruses is the organisation of their respective genomes. The genus *Pomovirus* represents a group of viruses exhibiting a tripartite genome (Arif and Reavy, 2000) whereas both the furoviruses (Verchot-Lubicz, 2003) and tobraviruses (Goulden *et al*., 1991) have bipartite genomes. The genome of the benyviruses is divided into four or five segments (Ward *et al*., 2007).

PMTV particles are rod-shaped, 18-20 nm in diameter and of two predominant lengths, 100-150 nm and 250-300 nm (Kallender *et al*., 1990). The genome consists of three single-stranded, positive sense RNA molecules (Scott, 1994; Torrance *et al*., 1999). RNA 1 (6.5 kb) (Savenkov *et al*., 1999) is estimated to comprise approximately half of the PMTV genome (Torrance *et al*., 1992) and is thought to be responsible for virus replication (Savenkov *et al*., 1999). RNA 2 (3 kb) (Scott *et al*., 1994) encodes a cysteine rich protein which may function to increase virulence or suppress the host defence mechanism (Lukhovitskaya *et al*., 2005). RNA 2 also encodes the triple gene block proteins (TGB), a triplet of proteins which many plant viruses utilise to facilitate spread and establishment in the host plant (Haupt *et al*., 2005). RNA 3 (2.5 kb) (Scott *et al*., 1994) encodes two proteins, the coat protein which is thought to be involved in foliar symptom expression (McGeachy and Barker, 2000) and a read-through protein which may be involved in vector transmission (Germundsson *et al*., 2002; Kashiwazaki *et al*., 1995). Experiments by Cowan *et al.* (1997) showed that the read-through protein is located at one end of the virus particle. The significance of the localisation of the read-through protein is not known; however, it is thought to be involved in attachment to the vector membrane to assist movement of the virus particles between the cytoplasm of the host plant and that of the vector (Adams *et al*., 2001). Indeed, Sandgren *et al*., (2001) reported deletions in the read-through domains of isolates maintained in the laboratory by manual inoculation as the virus no longer required the vector for transmission.

There are a number of isolates of PMTV: PMTV-T has been maintained by manual transmission in the laboratory since 1970 and is more virulent than most other isolates (Harrison and Jones, 1970); PMTV-S and -SW are field isolates from Scotland (Arif *et al*., 1995) and Sweden (Sandgren *et al*., 2001), respectively. There are also a number of Danish (Pečenková *et al*., 2004; Nielsen and Nicolaisen, 2003), Czech (Čeřovská *et al*., 2007) and Peruvian isolates (Mayo *et al*., 1996). Nucleotide sequencing has shown that the coat protein sequence is highly conserved (Reavy *et al*., 1997) and that the field and laboratory isolates differ in the read-through protein with the T isolate exhibiting a shorter read-through sequence (Reavy *et al*., 1998; Sandgren *et al*., 2001). Isolates of PMTV differ in their transmissibility by *S. subterranea*. Reavy *et al.* (1998) successfully acquired and transmitted the PMTV-S isolate (Scottish field isolate) using a mono-culture of *S. subterranea*; however, the same culture was unable to acquire and transmit the PMTV-T isolate. This may be explained by deletions in the read-through

sequence encoded by the RNA 3 molecule, which is thought to be involved with transmission (Reavy *et al*., 1998; Sandgren *et al*., 2001). Nielsen and Nicolaisen (2003) recently compared the sequence of a region encoding the read-through protein of RNA 3, in 9 Danish field isolates of PMTV. The isolates were tested for symptom expression using bait plants of three species (*C. amaranticolor*, *N. benthamiana* and *N. debneyi*). These isolates were grouped together according to symptom expression on the host plant; weak, medium and strong symptoms. However, no correlation between symptom expression and genotype or geographic origin was observed. It is yet to be determined whether the isolates differ with regard to virulence in potato.

In another study investigating differences in the various isolates of PMTV, ten isolates of PMTV were compared with isolate - T (Harrison and Jones, 1970). Symptoms of PMTV infection were observed in host plants of the *Solanaceae*, *Chenopodiaceae* and *Aizoaceae* families and PMTV infection was confirmed by sap inoculation to *Chenopodium amaranticolor* or tobacco. Symptoms in the host plants ranged from necrotic or chlorotic spots to concentric chlorotic or necrotic rings. Symptoms produced by inoculation with isolate - T were more obvious than those observed after infection by the other isolates. This study also highlighted the effect of environmental conditions on PMTV infection in *C. amaranticolor* and *N. tabacum* with symptoms of infection absent or inconspicuous in both species in the summer and obvious symptoms of infection in the winter months (c. 15ºC). Systemic infection of these host plants only occurred between December and March.

1.4.2 Transmission

Following a brief initial report that *S. subterranea* might be the vector for PMTV (Calvert and Harrison, 1966), the first evidence that PMTV was transmitted by *S. subterranea* was provided by Jones and Harrison (1969). Specifically, bait plants of *N. debneyi* and *N. tabacum* became infected with PMTV following exposure of their roots to PMTV-infected sporeballs (Jones and Harrison, 1969). However, these findings were not conclusive as there was a possibility that other fungal vectors in the soil may have been responsible for virus transmission. Arif *et al*., (1995) subsequently confirmed that *S. subterranea* was a vector for PMTV by using a mono-culture to acquire PMTV from the roots of infected bait plants. This viruliferous culture was then used to transmit the

virus to the roots of healthy bait plants. It is now widely accepted that *S. subterranea* is the only known vector for PMTV (Kirk, 2008).

The zoospores of *S. subterranea* are of particular importance as these are responsible for transmission of PMTV to potato plants (Merz, 1992). Infection occurs when zoospores which are carrying the virus penetrate the potato root tissue, stolons, young shoots and tubers[2] (Hims and Preece, 1975). The primary zoospores acquire virus particles of PMTV when *S. subterranea* develops in virus-infected host cells; however, the exact mechanism of acquisition remains uncertain (Germundsson *et al*., 2002). PMTV resides in resting spores of the powdery scab pathogen and can therefore remain infective in field soils for a long period of time (Germundsson *et al*., 2002). Calvert (1968) reported that a field remained infested with PMTV 18 years after the last crop of potatoes. PMTV can be spread to new sites if seed tubers with powdery scab containing viruliferous resting spores are planted (Jones and Harrison, 1969).

Environmental factors influencing infection by PMTV are generally considered to be those required for *S. subterranea* infection of potato. High soil moisture to allow zoospores to germinate and swim to the host is essential for infection. Consequently, the occurrence of PMTV is strongly associated with irrigation during the early stages of the growing season (Adams *et al*., 1986). High soil moisture is particularly conducive to infection during tuber initiation (Kirk, 2008). In Scotland, the incidence of PMTV is more commonly related to areas with an annual rainfall of over 760 mm/year with the probability of infection significantly increasing with rainfall of over 1140 mm/year, irrespective of soil type (Cooper and Harrison, 1973).

PMTV spreads irregularly within the plant and both diseased and symptomless stems may be observed (Calvert, 1968; Torrance *et al*., 1999). Only a proportion of the progeny tubers from an infected plant with haulm symptoms carry the infection and this can lead to a gradual elimination of the virus over a number of generations in the absence of its vector (Calvert, 1968; Cooper *et al*., 1976; Torrance *et al*., 1999). Therefore, PMTV in a stock of seed potatoes may be eliminated after several generations. It is important to note that all of these studies measure infection through the expression of symptoms, and do not take account of symptomless infection in either plants or tubers, nor of symptoms caused by other organisms e.g. *Tobacco rattle virus*

[2] The lifecycle of *S. subterranea* is illustrated in Figure 6, Section 1.3.1.1.

(TRV). Equally, Latvala-Kilby *et al.* (2009) reported PMTV infection but no symptoms of infection in 90% of tubers of cv. Kardal, 44% cv. Saturna, 95% cv. Bintje and 63% cv. Van Gogh, indicating that visual inspection for spraing symptoms may not detect a large proportion of infected tubers.

1.4.3 Symptoms and Consequences of Infection

With primary infection, i.e., from the soil-borne *S. subterranea*, PMTV typically produces slightly raised lines and rings (Figure 1-14) on the surface and/or brown arcs and lines (spraing) in the flesh of tubers (Figure 1-15) (Calvert & Harrison 1966; Harrison & Jones, 1970; Kurppa, 1989a).

Figure 1-14. External spraing symptoms (cv. Nicola).

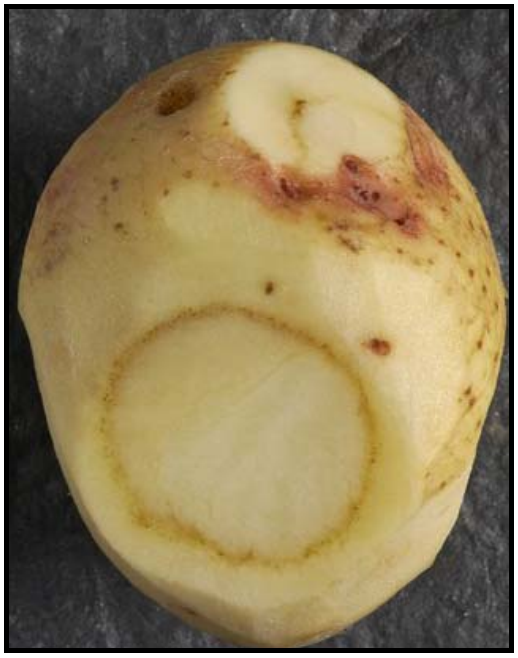

Figure 1-15. Tuber showing spraing symptoms (unknown variety).

Spraing occurs at the boundary of virus invasion (Jones, 2007); however, it does not prevent the virus from spreading throughout the tuber flesh (Jones, 1988a). Spraing is rarely seen at harvest and normally develops during storage due to changes in temperature. External spraing symptoms are occasionally observed in tubers near the soil surface (Harrison and Jones, 1970; Harrison and Jones, 1971a), possibly as a result of fluctuations in temperature. Harrison and Jones (1971a) found that, after 4 weeks storage at a constant temperature of either 5ºC or 13ºC, approximately 50% of tubers of cv. Arran Pilot developed dark rings on the surface of the tubers and in the tuber tissue. Symptoms were more severe at 13ºC than at 5ºC. Neither external nor internal spraing were observed on tubers stored at 18ºC for 4 weeks; however, storage at 18ºC for 2 weeks followed by 2 weeks at either 5ºC or 13ºC increased the incidence of both external and internal spraing symptoms. Eighty percent of tubers stored at 18ºC for 2 weeks followed by 2 weeks at 13ºC developed both internal and external spraing symptoms compared to 40% (external symptoms) and 20% (internal symptoms) when stored at 5ºC for 2 weeks following 2 weeks at 18ºC. A study by Mølgaard and Nielsen (1996), on the effect of post harvest temperature treatments on the development of spraing symptoms in stored tubers, reported that spraing symptoms significantly increased if tubers were stored at 18ºC for one week followed by storage at 8ºC for a further week compared with long term storage at 4ºC. The percentage increase ranged

from 33% (cv. Folva) to 48% (cv. Primula). Storage at 4ºC for two weeks also increased the incidence of spraing compared to that observed at harvest; however, the differences were not as great, the highest increase being 19% (cv. Primula). Kurppa (1989a) also reported a significant increase in spraing symptoms in infected tubers of cvs Olympia, Sabina and Saturna following the 18ºC/8ºC temperature treatment. It is important to note that most of these studies were conducted with small numbers of tubers and contradictions were evident between results reported by different authors.

Sokmen *et al.* (1998) compared the incidence of spraing symptoms in tubers grown in infested soil with results from ELISA testing for PMTV. Results varied with cultivar; symptoms correlated strongly with PMTV infection in cvs Pentland Marble, Arran Pilot and Brodick with 80%, 65% and 60% of infected tubers showing symptoms of infection respectively. Latent infection was more frequent in cvs Saturna and Pentland Crown in which only 10% and 8% of infected tubers had spraing.

Foliar symptoms of PMTV infection only occur if the plants are derived from infected seed tubers (Calvert, 1968; Cooper and Harrison, 1973; Harrison, 1974). Furthermore, symptoms of PMTV infection present in the early stages of plant development can, in some cases, gradually disappear in later stages of growth (Calvert, 1968; Kurppa, 1989b). Plants grown from PMTV-infected tubers produce three types of foliar symptoms: pale green chevrons on the leaves, bright yellow blotches (aucuba), rings and, mottling and/or a shortening of the internodes resulting in a bunching effect, or 'mop-top' (Calvert, 1968). The yellowing foliage symptoms can be confused with those induced by *Potato aucuba mosaic virus*, *Alfalfa mosaic virus*, and *Tomato black ring virus* or *Tobacco rattle virus* (Jones, 1988b). Foliar symptoms vary amongst cultivars and can also differ amongst plants of the same cultivar in the same area (Cooper and Harrison, 1973). Often, one type of foliar symptom can predominate, e.g., aucuba in cv. Craigs Royal; chevrons in cvs Craigs Alliance and King Edward; and mop-top in cv. Alpha (Calvert, 1968). Certain varieties can exhibit multiple foliar symptoms e.g. cv. Arran Pilot where chevrons, aucuba and 'mop-top' have been reported (Calvert, 1968).

Symptoms on secondarily infected tubers, i.e., tubers derived from infected plants, differ from tuber symptoms produced by primary infection. Secondary symptoms also vary with cultivar and include reticulate cracking (sometimes referred to as elephant hide blemish) and large primary cracks resulting in malformed tubers (Figure 1-16);

(Calvert and Harrison, 1966). Calvert (1968) observed more severe secondary symptoms in cultivars which develop the stunted mop-top symptom in the haulm, e.g., cvs Arran Pilot, Pentland Crown and Alpha.

Infection with PMTV has been reported to cause yield losses in cvs Arran Pilot, Arran Consul, Alpha, Saturna, Ostara and Bintje (Calvert, 1968; Kurppa, 1989a; Sandgren *et al*., 2002). Yield loss is due to a decrease in tuber numbers rather than a decrease in tuber size (Calvert, 1968; Kurppa, 1989a). This decrease can be substantial, with Kurppa (1989a) reporting a 37% decrease and Calvert (1968) a 26% reduction in yield. However, both of these studies were small scale; Calvert (1968) only measured the yield from 10 plants in each of 4 replicates. These studies also took no account of symptom development on the plant derived from infected seed. Nielsen and Mølgaard (1997) reported no difference in the yield of crops of cv. Saturna with severe spraing compared to those with slight or no spraing. However, this is perhaps not surprising as most of this PMTV infection would have been primary infection and therefore infected plants would be symptomless.

Figure 1-16. Secondarily infected tubers of cv. Cara.

PMTV-induced spraing is of particular importance in the Nordic countries (Kurppa, 1989b, Nielsen and Mølgaard, 1997). Nielsen *et al.* (2003) concluded that PMTV-induced spraing is one of the single most important problems in the Danish potato

industry, especially in tubers produced for processing, as crops with spraing symptoms would be rejected by processors (Kurppa, 1989a; Nielsen and Mølgaard, 1997). A survey by Nielsen and Mølgaard (1997) further showed that both PMTV and *S. subterranea* are widespread in all of Denmark's key potato growing areas.

A survey of Scottish seed potato stocks conducted in 1971-1972 by Cooper and Harrison (1973) revealed symptoms of PMTV infection in both the haulm and daughter tubers in 46% of the 224 crops studied; however, the survey was based on visible symptoms and did not take account of symptomless infection or infection by other organisms e.g. TRV. Sokmen *et al.* (1998) reported the occurrence of symptomless infection in some cultivars, indicating that PMTV infection may be missed if tests are based solely on visual symptoms.

1.4.4 Detection and Control

1.4.4.1 Detection

Field inspection of plants or tubers is generally considered inconclusive for the accurate diagnosis of PMTV infection. This is because:

i) TRV-induced spraing is indistinguishable from PMTV-induced spraing (Kurppa, 1989a)
ii) Symptomless infection of tubers may occur (Harrison and Jones, 1971a)
iii) Symptomless infection of plants may occur and infection of plants from soil inoculum will not cause plant symptoms in that year (Calvert and Harrison, 1966; Sokmen *et al*., 1998)

The true amount of infection can therefore only be determined by testing for the virus in the laboratory. Early tests involved bioassays using indicator plants, e.g., *Chenopodium amaranticolor* which developed symptoms when infected by PMTV. Although informative, these tests were time consuming (taking in excess of 14 days for symptoms to develop) and were often unreliable (Calvert and Harrison, 1966; Jeffries, 1998). With the development of antibody based assays (Enzyme Linked Immunosorbant Assay - ELISA), it became possible to detect PMTV in two days. ELISA can be unreliable, however, and taking into account the uneven distribution of virus in the growing plant (Calvert, 1968; Torrance *et al*., 1999), there is therefore, a possibility of false negative results.

A study by Sokmen *et al.* (1998) investigated infection in the growing plants derived from PMTV infected seed tubers, as detected by ELISA. PMTV was detected in approximately one third of the plants grown from infected seed; however, this study did not record if symptoms were visible. Also in this study, a few tubers which had tested negative for PMTV infection produced plants which tested positive for PMTV, indicating that the virus titre in the seed tubers may have been below the limit of detection. However, in a separate study, Arif and Torrance (1996) concluded that direct tuber testing for PMTV is a reliable method of detection. This study compared detection of PMTV when sampling the tissue from either the rose or stolon end of the tuber in 3 cultivars. For cv. Pentland Marble, the virus was detected in all of the infected tubers by sampling either the rose or stolon end whereas for cv. Arran Pilot, sampling from the rose end was more effective at virus detection than sampling from the stolon end; however, for cv. Pentland Crown, the virus was more concentrated at the stolon end than at the rose end. Therefore, combining tissue from both the rose and stolon end of the tuber was considered the most effective way to increase the likelihood of virus detection.

The exploitation of nucleic acid based techniques has led to the development of highly sensitive detection methods. Reverse-Transcriptase PCR (RT-PCR) is a particularly sensitive detection method. Boonham *et al.* (2000) developed a multiplex PCR assay to discriminate between spraing caused by PMTV and spraing caused by TRV infection and compared the efficacy of detection with ELISA. They reported a 10,000 fold increase in sensitivity compared to the ELISA test; however, few details were given of the methodology used. Arif *et al.* (1994) compared the reliability of ELISA and RT-PCR in detecting PMTV in the roots and leaves of bait plants of *N. debneyi*. RT-PCR detected PMTV in both the leaves and roots of the bait plants after 3 weeks' growth in the soil whereas ELISA only detected the virus after 5 weeks and symptoms of PMTV infection were only visible in the bait plants of *N. debneyi* after 6 weeks' growth in PMTV infested soil. Although RT-PCR is more sensitive, sample preparation for this method is time consuming. ELISA is therefore considered by many to be the cheapest and most convenient method for routine analysis of large sets of samples.

Both ELISA and RT-PCR rely on the presence of RNA 3, which encodes the coat protein (McGeachy and Barker, 2000), as this is a highly conserved region of the PMTV

genome. There is, however, evidence that RNAs 1 and 2 can accumulate and spread systemically in infected plants in the absence of RNA 3 and that these plants do not display symptoms (McGeachy and Barker, 2000).

1.4.4.2 Control

Control of PMTV is hampered by our incomplete knowledge of its epidemiology, making integrated control strategies the most suitable approach to control. Limited control of PMTV may be achieved by roguing plants with haulm symptoms. Calvert (1968) showed that the incidence of foliar symptoms was lower on plants derived from seed tubers with no spraing than those from spraing affected seed. Studies have shown that roguing symptomatic plants of cvs Arran Pilot and Red Craigs Royal can reduce the incidence of PMTV in a crop (Cooper *et al*., 1976). However, roguing is not an entirely reliable control measure as asymptomatic plants will remain in the field. Removing seed tubers with external symptoms of spraing prior to planting can also reduce the incidence of PMTV infection in the daughter crop (Calvert, 1968). Harrison and Jones (1971a) also recommended storing seed tubers in conditions conducive to spraing development, enabling affected tubers to be discarded.

Cultivar selection can also help minimise PMTV infection in a crop. Cultivars vary in their sensitivity to PMTV infection, e.g. cv. Saturna, widely used in the Scandanavian potato-processing industry, is very sensitive (Sandgren, 1995; Nielsen & Mølgaard, 1997), cvs Bintje, Pito, Hertha, and Record are tolerant, i.e. infected tubers are symptomless and cvs Appell and Desirée are more resistant to PMTV infection (Germundsson *et al.*, 2002; Kurppa, 1989a).

Control measures for PMTV are generally targeted at its vector *S. subterranea* (see Section 1.3.1.3). Planting clean seed and avoiding potato cultivation in soils infested with virus-carrying *S. subterranea* will minimise the risk of infection. Ensuring that the seed tubers are not contaminated with viruliferous sporeballs will also prevent the spread of the pathogen to new sites. However, sporeballs on tubers may not be visible to the naked eye resulting in the possibility of unwittingly selecting contaminated tubers**.**

1.5 Project Aims

The primary aim of this project is to contribute to the knowledge base for the epidemiology of *Potato mop-top virus*. Within this broader aim, the research has a number of more specific objectives:

1. To profile the distribution of *Potato mop-top virus* throughout the main seed potato producing areas of Scotland, with the aim of clarifying the level of risk this pathogen poses to the Scottish seed potato industry.

2. To assess the importance of seed borne inoculum in the transmission of PMTV from seed to daughter tubers.

3. To determine the variability in susceptibility amongst cultivars in terms of both PMTV infection and symptom expression in tubers and the growing plant.

4. To evaluate the importance of soil-borne inoculum in causing economic outbreaks of PMTV in relation to site and temperature.

Chapter 2. Incidence of PMTV in Scottish seed crops

2.1 Objectives

In 2004, a stratified survey was conducted to determine the occurrence of PMTV in seed potato crops of four commonly grown cultivars in the major seed potato producing areas of Scotland. The data would also provide evidence on the extent of differences in susceptibility of cultivars to PMTV infection and the incidence of symptomless infection.

2.2 Materials and Methods

2.2.1 Survey sample selection

Seed potato crops of Super Elite (SE) class of four commonly grown potato cultivars were selected from the 2004 Scottish Seed Potato Register, from 16 counties representing four seed producing regions in Scotland (Table 2-1 and Figure 2-1). The cultivars were Hermes, Maris Piper, Nicola and Saturna, occupying 10%, 20%, 2.5% and 2.8% of seed area respectively (Table 2-2). Sampling was confined to SE seed potatoes which occupied 82% of total seed area. This allowed a balanced number of samples of the same class to be taken for each cultivar and region. The cultivars were amongst the 10 cultivars most commonly grown at SE grade, by area, in Scotland in 2004 (Scottish Seed Potato Register, 2004) (Table 2-2).

Table 2-1. The 4 major seed producing regions of Scotland and their component counties.

Region	County
North-Eastern	Aberdeenshire, Banff and Kincardine
Central	Angus, Perth and Fife
Northern	Caithness, Inverness, Moray, Nairn, Ross and Sutherland
The Borders	Berwick, East Lothian, Dumfries and Roxburgh

Table 2-2. Top 10 cultivars classified at inspection as class Super Elite in Scotland in 2004 (Results based on total area; data from the Scottish Seed Potato Register 2004).

Cultivars	Total area (Hectares)
Maris Piper	1951.3
Desiree	933.6
Hermes	910.5
Maris Peer	621.0
Pentland Dell	494.5
Estima[3]	345.9
Saturna	274.4
Russet Burbank	261.4
Nicola	246.5
King Edward	241.2

Ten crops of each of the four cultivars were selected from each region at random, with the exception that no more than one crop was selected from a farm, if possible. However, in several cases it was not possible to obtain 10 crops of each cultivar from the same region; in which case, all available crops of the cultivar in that region were sampled. A sample of 200 seed sized tubers (35-55 mm) was collected at random by inspectors from the Scottish Government, Rural Payments and Inspections Directorate, sealed in new paper sacks and dispatched to SASA. Tubers were stored at 4ºC until all samples had been received. A total of 128 crops were sampled.

[3] Classification data for cv. Estima was not included in the Scottish Seed Potato Register 2004 as crops had to be post-harvest tested for virus before final acceptance. Unpublished data obtained from the Scottish Seed Potato Classification Scheme (SSPCS) unit at SASA.

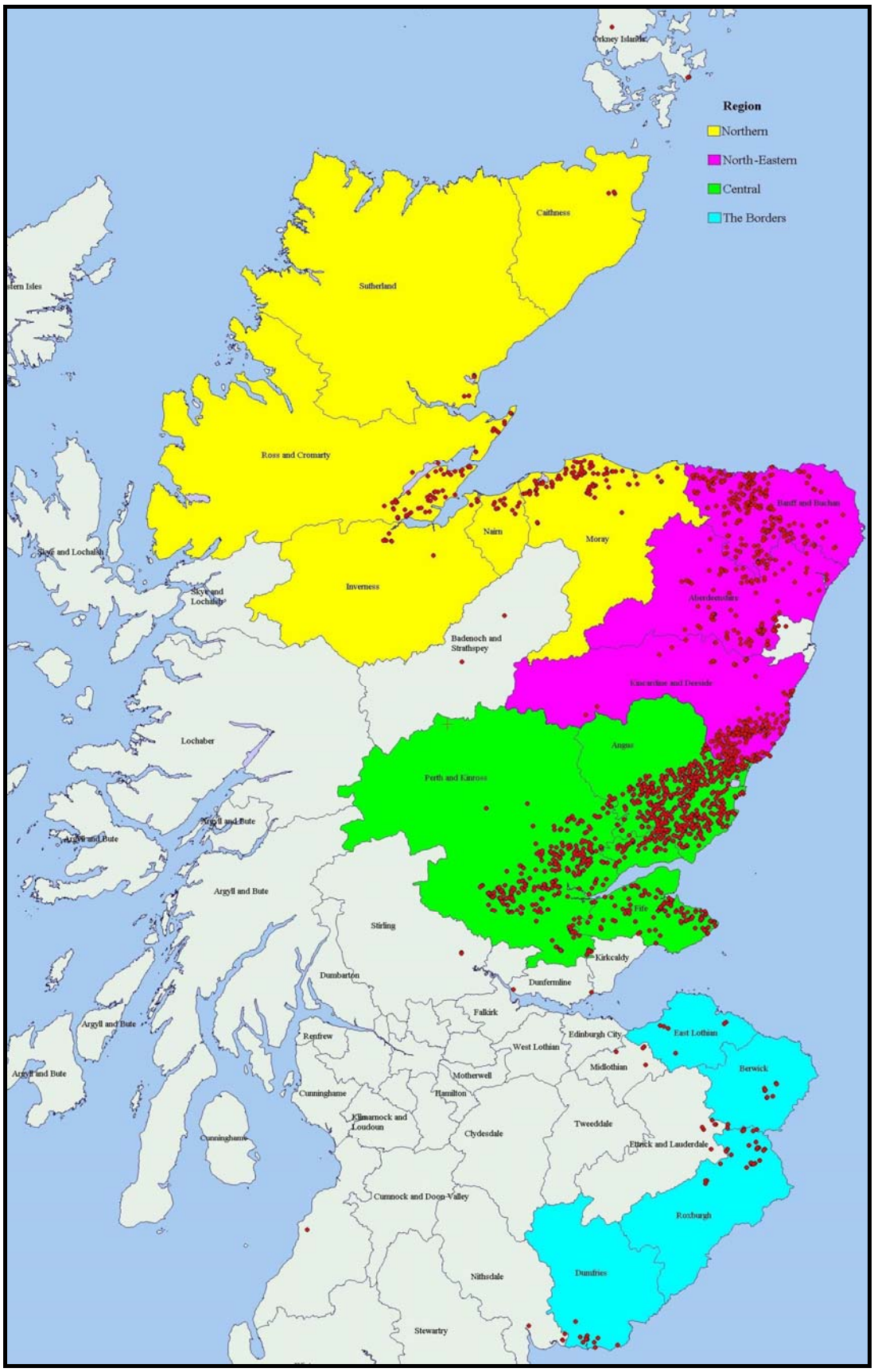

Figure 2-1. The four major seed producing regions and counties from which crops were sampled. Red dots denote a seed potato crop. Source: SASA – Scottish Seed Potato Classification Scheme (2002-2007).

2.2.2 Storage for spraing development

Tubers were stored in conditions conducive to spraing development (Harrison and Jones, 1971a; Kurppa, 1989a; Mølgaard and Nielsen, 1996; Sokmen, *et al.*, 1998). The bags of tubers were stacked on wooden pallets and stored in the dark at 18°C (±2°C) for two weeks. The temperature was then lowered to 4°C (±2°C) for a further week.

2.2.3 Spraing assessment

All tested tubers were sliced longitudinally down the centre of the tuber and once longitudinally on either side of the first slice to give six 'faces' of the tuber for examination (Figure 2-2). Results were recorded as a percentage of spraing in the stock.

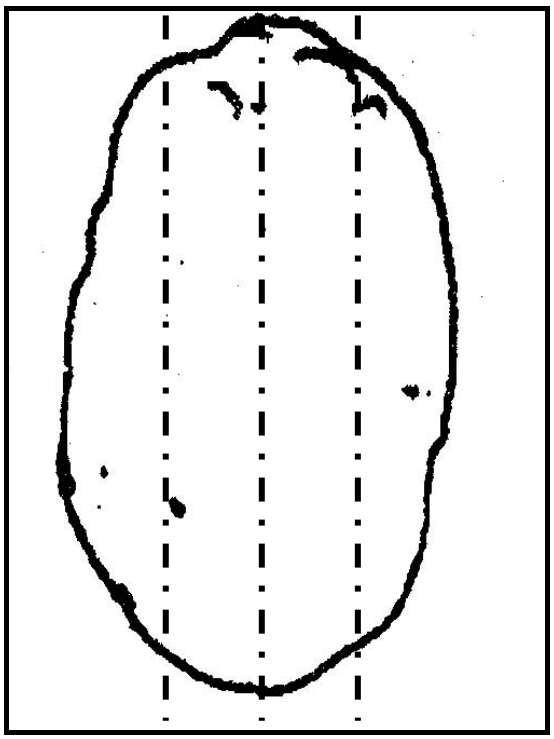

Figure 2-2. Schematic of cutting of potato used for spraing assessment. Dotted lines indicate how tubers were sliced (Anon, 1976).

2.2.4 Double antibody sandwich enzyme-linked immunosorbant assay (DAS-ELISA)

The double antibody sandwich enzyme-linked immunosorbant assay (DAS-ELISA) method for the detection of PMTV was carried out according to the standard operating procedures in place at SASA, as described below. One hundred and fifty tubers from each of the 128 stocks were tested.

2.2.4.1 Sample preparation

Cores of tuber tissue were taken from the rose and heel end (stolon attachment point) of individually numbered tubers. The core was removed using a No. 2 cork borer which was sheathed in Nalgene tubing 3cm from the tip to give a standard depth of tissue and was rinsed in water after coring each tuber to prevent cross-contamination. The two cores were placed into the back of a numbered Bioreba homogenisation bag (Bioreba AG, CH). Five millilitres of tuber extraction buffer (Appendix 1) was added and the samples were ground using a Homex 5 homogeniser (Bioreba AG, CH).

2.2.4.2 Coating plates for ELISA testing

All ELISA tests were carried out using 96-well, polystyrene microtitre plates (Nunc, DK) that had been precoated with a PMTV specific monoclonal antibody (SASA Antibody Unit). Coating antibody was diluted to 1mg/mL in coating buffer (Appendix 1) and 200µl was dispensed into each well. Plates were incubated at 37°C for 4 hours or overnight at 4°C and then washed twice using concentrated PBST (Appendix 1). The plates were then inverted and patted dry on tissue paper to remove excess moisture. The PMTV plates were either used immediately or placed in a polythene bag and stored at -20°C until required.

2.2.4.3 ELISA procedure

Two hundred microlitres of homogenised samples were used in each assay and each sample was analysed in duplicate. Tuber samples from positive and negative control material were also added to each plate. Negative control tubers were healthy tubers that were continually grown in the glasshouses by the Virology and Zoology Section, SASA. Positive control material was tuber tissue that had previously tested positive for PMTV by ELISA. The material was maintained freeze-dried by the Virology and Zoology Section, and reconstituted in tuber extraction buffer when required. Following overnight incubation at 4°C, plates were washed with 0.1M PBS containing 0.5%

Tween-20 (aq., v/v). Two hundred microlitres of a PMTV-specific alkaline phosphatase conjugate (250mg/mL) was added to each well and plates were incubated for a further 2 hours at 37°C. Plates were then washed three times with 0.1M PBS containing 0.5% Tween 20 (aq., v/v) prior to the addition of substrate. Wells were loaded with 200µl of p-nitrophenyl phosphate (pNPP) substrate (1mg/mL, aq.) and plates were incubated at room temperature in the dark for 1 hour to allow colour development. Absorbance was assessed using a microplate reader (Dynatech Laboratories, Alexandria, VA, USA) at λ 405nm. Absorbance at λ 620nm was also measured as a reference for background absorbance. This assay is non quantitative; samples were deemed to be positive when the mean values were at least twice as great as those of the negative controls.

2.2.5 Statistical analysis

Statistical analyses of the experimental data were carried out using the Genstat statistical package (release 8.1, Lawes Agricultural Trust, 2005). Analysis of the effects of region and cultivar on PMTV infection and expression of spraing symptoms was conducted using a binomial generalised linear model using the logit link function. Logistic regression deals with categorical variables with two possible outcomes i.e., the presence or absence of PMTV infection, and was therefore appropriate for these data. Treatments in which the frequency of disease was zero or close to zero were excluded form analysis. Logistic regression provided the additional benefit of incorporating the original sample size in to the probability estimation, and thus taking into account differences between the numbers of crops sampled between regions. Differences were considered significant at the $P<0.05$ level.

2.3 Results

The incidence of PMTV in tubers of crops of cvs Hermes, Maris Piper, Nicola and Saturna is presented in Table 2-3 (Raw data are presented in Appendix 2).

Table 2-3. Incidence of crops with PMTV tuber infection in relation to region of production and cultivar.

	Cultivar				
Region	**Hermes**	**Maris Piper**	**Nicola**	**Saturna**	**Mean %**
North-Eastern (Aberdeenshire, Banff & Kincardine)	0/6	3/8	6/9	1/7	33%
Central (Angus, Perth & Fife)	7/10	5/10	7/10	5/11	59%
Northern (Caithness, Inverness, Moray, Nairn, Ross & Sutherland)	3/11	2/13	3/7	2/5	28%
The Borders (Berwick, East Lothian, Dumfries & Roxburgh)	1/8	3/9	0/1	0/3	19%
Mean %	31%	33%	59%	31%	**Total = 38%**

Of the 128 crops tested, PMTV infection (Table 2-3) was detected in 48 (38%) crops. Spraing symptoms were observed in 24 (19%) (Table 2-4) but PMTV-infected tubers were detected in only 16 of these crops, indicating that not all spraing symptoms were attributable to PMTV. In addition, spraing was not observed in 32 out of 48 crops infected with PMTV suggesting that symptomless tuber infection by PMTV can be common. The highest incidence of PMTV infection (82%) and spraing (26%) occurred in different crops of the cultivar Nicola (data not shown). Overall, the mean incidences of crop infection in the other three cultivars were similar (31-33%) (Table 2-3), though more spraing symptoms were observed in infected crops of cv. Saturna than either cv. Hermes or cv. Maris Piper (Table 2-5).

The proportion of crops infected by PMTV was greater for the Central region of Scotland than the other regions, particularly Northern Scotland and The Borders. The incidence of PMTV tuber infection in crops differed significantly ($P = 0.005$) amongst

regions (Table 2-3); however, there was no difference amongst the regions in the proportion of crops affected by spraing (P = 0.139) (Table 2-4). Furthermore, neither the incidence of crops with PMTV tuber infection nor those with spraing differed significantly with cultivar (P = 0.058 and 0.075 respectively) (Tables 2-3 and 2-4).

Table 2-4. Incidence of crops with spraing in relation to region of production and cultivar.

	Cultivar				
Region	**Hermes**	**Maris Piper**	**Nicola**	**Saturna**	**Mean %**
North-Eastern (Aberdeenshire, Banff & Kincardine)	0/6	0/8	1/9	1/7	7%
Central (Angus, Perth & Fife)	3/10	1/10	4/10	4/11	29%
Northern (Caithness, Inverness, Moray, Nairn, Ross & Sutherland)	1/11	4/13	3/7	0/5	29%
The Borders (Berwick, East Lothian, Dumfries & Roxburgh)	0/8	0/9	1/1	1/3	10%
Mean %	11%	13%	33%	23%	**Total = 19%**

Table 2-5 Incidence of spraing in PMTV-infected crops in relation to region of production and cultivar.

	Cultivar				
Region	**Hermes**	**Maris Piper**	**Nicola**	**Saturna**	**Mean %**
North-Eastern (Aberdeenshire, Banff & Kincardine)	0/0	0/3	1/6	1/1	20%
Central (Angus, Perth & Fife)	3/7	1/5	3/7	4/5	46%
Northern (Caithness, Inverness, Moray, Nairn, Ross & Sutherland)	0/3	1/2	2/3	0/2	30%
The Borders (Berwick, East Lothian, Dumfries & Roxburgh)	0/1	0/3	0/0	0/0	0%
Mean %	27%	15%	38%	63%	**Total = 33%**

Overall, spraing symptoms in PMTV infected crops were more common in Central Scotland, with 46% of PMTV-infected crops grown in this region showing spraing symptoms, compared to just 30% and 20% of PMTV-infected crops grown in the Northern and North Eastern areas of Scotland respectively (Table 2-5). Spraing symptoms were not observed in any of the four PMTV-infected crops grown in the Borders. Spraing symptoms were observed in cv. Saturna more frequently than in the other cultivars (63%), indicating that this cultivar is more likely to express symptoms of infection than the other cultivars in this study.

For all cultivars, the incidence of PMTV in the tubers did not correlate with that of spraing (Figure 2-3). Generally, the incidence of PMTV-infected tubers was greater than the incidence of spraing affected tubers in a crop. For example, one crop of cv. Maris Piper grown in Northern Scotland had 0.7% tubers affected by spraing but 23% of tubers infected with PMTV. In contrast, another crop of cv. Maris Piper produced in Central Scotland had 0.7% PMTV infection but a significantly higher incidence of tubers were affected by spraing (18%).

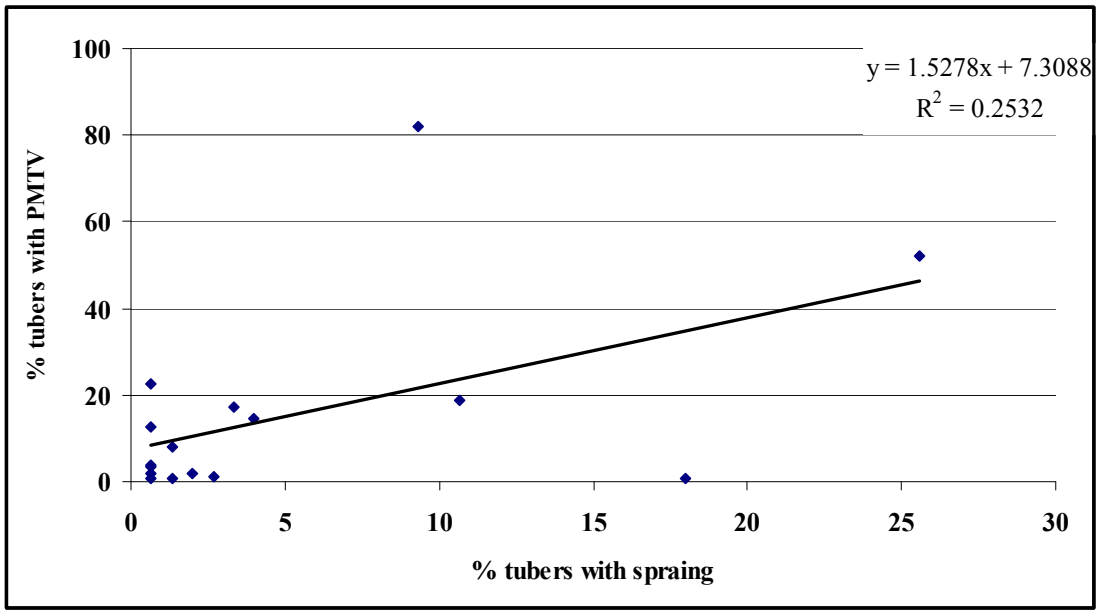

Figure 2-3. A scatter plot illustrating the relationship between PMTV infection and spraing symptoms for crops in which both PMTV and spraing were detected.

2.4 Discussion

The results of the survey clearly indicate that PMTV infection occurs in all of the major seed growing regions of Scotland; being detected in 38% (48 out of 128) of all the Scottish seed crops tested. Despite PMTV being widely distributed in Scotland, the incidence of tuber infection by PMTV in Scottish seed crops is generally low, with only 11% of the seed stocks examined exhibiting virus infection greater than 4% (maximum collective tolerance for Basic seed potatoes produced in Scotland for marketing within the EU; includes rots and surface diseases).

A previous small-scale study by Jones and Harrison (1972) reported a somewhat higher incidence of PMTV infection in Scottish crops, with foliar symptoms of PMTV being observed in 65% of crops of cvs Arran Pilot and Red Craig's Royal; this study also reported a low incidence of PMTV foliar symptoms within individual crops, with only 11% of the crops having more than 5% of plants with foliar symptoms. However, the figures quoted in this earlier study may not reflect the true extent of PMTV infection in Scottish seed stocks as that survey was based solely on symptom expression in growing plants and some crops were not assessed until early August, by which time symptoms may have become less conspicuous. Furthermore, this study assessed the occurrence of PMTV on the basis of symptom expression in the growing plant and would therefore

not take account of primary infection i.e., infection of tubers from the soil in the year of planting or symptomless secondary infection. As the survey results presented in this section are based on direct tuber testing, which takes account of both primary and secondary infection, they are likely to represent a more accurate estimate of the current distribution of PMTV in seed crops in Scotland.

The incidence and distribution of PMTV in Scotland, as reported in this chapter, can be compared with that of a number of other countries where similar studies have been conducted. Specifically, studies in Peru (Salazar and Jones, 1975), Denmark (Nielsen and Mølgaard, 1997), the USA and Canada (Xu *et al*., 2004) and Costa Rica (Montero-Astúa *et al*., 2008) also showed PMTV to be widespread across potato producing regions. However, the actual incidence of PMTV within individual potato crops was not reported for most of these studies, with the exception of Costa Rica, where incidence of PMTV was typically less than 20%, with 100% infection detected in some cases. Direct comparison between studies is also difficult due to major methodological differences, which include differences in approaches to infection assessment and inconsistencies in sampling methods used for tissue analysis. Salazar and Jones (1975) and Nielsen and Mølgaard (1997) for example, used symptom based assessments (plants and tubers respectively) to determine the incidence of infection; however, this method could underestimate the actual incidence of infection due to the possibility of latent infection. The use of diagnostic testing would have allowed for greater accuracy in determining the level of infection in these cases. Xu *et al*., (2004) and Montero-Astúa *et al*., (2008) both used diagnostic approaches in their studies to address this issue; however, in both of these studies tuber tissue sampling was inconsistent. For example, for direct tuber testing by ELISA, Xu *et al*., (2004) sampled tuber tissue from the stolon end of dormant tubers and the rose end of tubers which had broken dormancy, while Montero-Astúa *et al*., (2008) sampled tuber tissue from the mid-region of tubers. However, Arif and Torrance (1996) have previously shown that sampling tuber tissue from both the rose and heel end for ELISA testing (as was the case for the study described in this chapter) increases the accuracy of detection due to the irregular distribution of the virus in tuber tissue.

The results reported in this chapter highlight significant differences in PMTV infection between regions and are similar to those reported by Cooper and Harrison (1973). Specifically, Cooper and Harrison (1973) reported symptoms of PMTV infection in

57% of the crops studied in Central Scotland, 35% in North Eastern and 34% in the Borders compared to 59%, 33% and 19% PMTV infection in the corresponding areas for this study[4].

The differences in PMTV infection amongst regions may be partly due to climatic differences, with lower average rainfall evident in the Borders (particularly to the East, where the majority of seed crops in this region are grown) (UK Meteorological Office, 2009), where the incidence of PMTV infection was lowest. Cooper and Harrison (1973) also reported that crops in Central Scotland are grown at higher altitudes than in the other areas. Studies in Peru (Salazar and Jones, 1975) and Costa Rica (Montero-Astúa *et al*., 2008) have also shown that higher incidences of PMTV infection are observed in fields located at higher altitudes. Salazar and Jones (1975) also reported that PMTV was not observed in coastal plantings, which constitute a large proportion of the potato crops grown in the Northern Scotland study area, and this may explain the lower incidence of PMTV infection in this area.

A further factor which may influence variability in the incidence of PMTV between (and within) regions is soil type. Nielsen and Mølgaard (1997) reported a higher incidence of PMTV infection in tubers grown in coarse sandy soil rather than fine grain sandy soil or fine grain sandy loam. Conversely, Cooper and Harrison (1973) and Jones and Harrison (1972) reported that the occurrence of PMTV was not affected by soil type. The range of infested soils identified by Jones and Harrison (1972) included heavy, medium and light loams, and sandy soils; PMTV was not detected in clay soil, however, only one clay soil sample was included in their study. The survey presented here did not investigate the effects of soil type on the occurrence of PMTV; however, the predominant general soil types across the four study areas are either brown earths, or humus-iron podzols (both of which are generally free-draining) (Towers *et al*., 2006), or a combination of the two; soil type, in this case, may not have been a major influence on the occurrence of PMTV.

These results clearly show that symptomless infection by PMTV in potato tubers is widespread in Scotland even although tubers were treated to encourage spraing

[4] The areas mapped by Cooper and Harrison (1973) are not identical to the areas studied in this chapter; however, they do correspond approximately. The area referred to by Cooper and Harrison (1973) as 'Far Northern' was not included in this study.

development. Overall, two thirds of the crops in which PMTV was detected were not affected by spraing symptoms, indicating that symptomless infection may be more common than symptomatic tuber infection. The presence of spraing is therefore unlikely to be a good indicator of the incidence of PMTV infection within crops of the four cultivars included in this study. As previous Scottish studies linked the presence of spraing symptoms to PMTV infection (Jones and Harrison, 1972; Cooper and Harrison, 1973), they may have either underestimated the incidence of PMTV (due to symptomless infection), or overestimated the incidence of PMTV (due to the presence of spraing caused by TRV or other physiological factors). Indeed, Dale and Neilson (2006) reported that spraing caused by TRV outnumbers spraing caused by PMTV by approximately 3:1 in Scottish samples studied from 2002-2006.

One factor that should be considered in interpreting the results described in this chapter is that an overall result for the incidence of PMTV and spraing was reported for each crop. Therefore, these results do not account for spraing symptoms caused by TRV infection, which suggests that a percentage of tubers with spraing symptoms may be infected with TRV rather than PMTV (or possibly both). Future work is required to determine the relative importance of PMTV and TRV infection in spraing development.

In this study, the incidence of PMTV was greater in cv. Nicola than for cvs Hermes and Maris Piper, as was the incidence of spraing. However, the incidence of PMTV-infected crops containing spraing affected tubers was greatest in cv. Saturna, which may indicate that the presence of spraing in tubers of cv. Saturna has the potential to be a reliable indicator of PMTV infection. These results are confirmed by a Danish study of 26 potato cultivars, in which cv. Saturna was found to be the most susceptible to spraing (Nielsen and Mølgaard (1997). These findings contradict a previous study by Sokmen *et al.*, (1998) in which the majority of PMTV infected tubers of Scottish-grown cv. Saturna were symptomless. However, the results reported here and in the study by Nielsen and Mølgaard (1997) were based on tubers grown under normal field conditions, whereas, the tubers in the Sokmen *et al.*, (1998) study were grown in an unheated screen house in artificially inoculated soil. Direct comparison between these studies is, therefore, difficult and potentially misleading.

The higher incidence of spraing detected in the Central region of Scotland could be attributed to the proportion of susceptible cultivars sampled in this region. A higher

proportion of crops tested in the Central region of Scotland were crops of cvs Nicola and Saturna, compared with the other regions of Scotland, where cvs Hermes and Maris Piper made up a greater proportion of the samples. This was particularly the case in the Borders, where spraing was not as prevalent as in Central Scotland. Spraing symptoms were observed in crops of both cvs Nicola and Saturna grown in the Borders; however, tubers from these crops were free from PMTV indicating that the cause of spraing may have been infection by TRV. Furthermore, the overall finding, in this study, that spraing was found more frequently in PMTV infected crops of cv. Saturna than the other cultivars was slightly at odds with the overall finding that cv. Nicola has a higher incidence of spraing. Clearly, in the case of cv. Nicola, symptoms are also likely to have been caused by factors other than PMTV infection, i.e., TRV.

Chapter 3. Seed Transmission Experiments - Health of seed at a common site

3.1 Introduction

These experiments were conducted in 2004 and 2005 with the objective of determining the extent of PMTV transmission from infected seed tubers to daughter tubers in a range of cultivars planted at a common site. These seed transmission experiments also provided information on the relationship between PMTV infection in seed tubers and symptoms in foliage and tubers, the detection of PMTV in the leaves of symptomatic and asymptomatic plants, and the effect of symptom development in seed tubers on the transmission of the virus to daughter tubers.

3.2 Materials and Methods

3.2.1 Seed Transmission Experiments 2004

3.2.1.1 Sample selection and experimental design

Four cultivars (cvs Cara, Maris Piper, Nicola and Slaney) susceptible to PMTV infection were selected for these experiments. Seed potatoes were obtained from crops identified during post harvest inspections in 2003 as having PMTV and confirmed as containing a high incidence of PMTV infection by ELISA. Tubers from each of the selected crops were tested to obtain 120 PMTV-infected tubers and 120 PMTV-free tubers of each cultivar, and planted on 18 May, 2004 at SASA's Gogarbank Farm. Unless otherwise stated, experiments were laid out in a randomised block design with 3 replications; each plot consisted of 4 drills, each of 10 tubers. Tubers were spaced 0.38m apart along the drills, which were 0.7m apart. Each plot was guarded lengthwise and across drills by cv. Edzell Blue which produces blue skinned tubers. Foliage was inspected for visual symptoms characteristic of PMTV infection during the growing season; any plants displaying symptoms were marked and recorded. Plants of a different variety and plants affected by other viruses and blackleg were marked and discarded at harvest. Leaves from symptomatic and asymptomatic plants were sampled and tested for PMTV by ELISA. The haulm was destroyed on 2 September, 2004 by applying diquat dibromide (Reglone®, Syngenta) at the rate recommended by the

manufacturer and daughter tubers were harvested three weeks later. At harvest, daughter tubers from symptomatic plants were harvested first by hand and placed in polypropylene sacks. The remaining daughter tubers from the asymptomatic plants were then harvested using a single row digger and hand lifting, and placed in separate polypropylene sacks. The yield of the daughter tubers from both symptomatic and asymptomatic plants was measured separately for each plot. Results are presented as total weight for each plant. Tubers were stored in conditions conducive to spraing development and assessed for symptoms as described in Sections 2.2.2 and 2.2.3. For each plot, all daughter tubers from symptomatic plants and 150 tubers from asymptomatic plants were tested for PMTV by ELISA as described in Section 2.2.4.

3.2.1.1.1 Experiment 1

The extent of PMTV transmission from seed to daughter tubers was examined in two cultivars (cvs Maris Piper and Nicola). Seed potatoes from two crops of cv. Nicola, originating from the same farm but different fields, were included in the experiment as both were found to have a high incidence of PMTV when tested. The randomised layout is shown in Figure 3-1.

3.2.1.1.2 Experiment 2

In this experiment, transmission of PMTV from seed to daughter tubers was studied in cv. Cara; a spraing sensitive cultivar. The aim of this experiment was to determine whether the presence of spraing in seed tubers influenced the movement of the virus to daughter tubers. Prior to planting, tubers which tested positive for PMTV by ELISA were sub-divided on the basis of the presence or absence of spraing symptoms in the tuber flesh. The experimental layout is shown in Figure 3-2.

3.2.1.1.3 Experiment 3

A third experiment comparing the transmission of PMTV from infected seed tubers was conducted with cv. Slaney. This cultivar was not included in Experiment 1 as there were insufficient PMTV-infected tubers for the design. The plot size was reduced to 20

tubers planted in 2 drills, each of 10 tubers. There were four replicate blocks. The experimental layout is shown in Figure 3-3.

3.2.1.2 Leaf sampling and testing by ELISA

For symptomatic plants, one leaflet was sampled from each of four compound leaves on the affected stems. For asymptomatic plants, one leaflet was sampled from four separate compound leaves on separate stems of the plant. The four leaflets were placed into the back of a Bioreba homogenisation bag (Bioreba AG, Switzerland). Five millilitres of leaf extraction buffer (Appendix 1) was added to the bag and the leaflets were ground using a Homex 5 homogeniser (Bioreba). A further 5mL of leaf extraction buffer was added to the bag after maceration. Samples were tested for PMTV by ELISA as described in Section 2.2.4.

	G		G		
G	N1/H 17	G	MP/H 18	G	
	G		G		
G	N1/I 16	G	MP/I 15	G	**Block 3**
	G		G		
G	N2/I 13	G	N2/H 14	G	
	G		G		
G	MP/H 12	G	N2/H 11	G	
	G		G		
G	N2/I 9	G	N1/H 10	G	**Block 2**
	G		G		
G	N1/I 8	G	MP/I 7	G	
	G		G		
G	MP/I 5	G	N1/H 6	G	
	G		G		
G	N1/I 4	G	N2/H 3	G	**Block 1**
	G		G		
G	N2/I 1	G	MP/H 2	G	
	G		G		

Figure 3-1. Experiment 1 Randomised block design layout of seed transmission experiment for cvs Maris Piper and Nicola. (N1/I=Nicola (crop 1) Infected; N1/H=Nicola (crop 1) Healthy; N2/I=Nicola (crop 2) Infected; N2/H=Nicola (crop 2) Healthy; MP/I=Maris Piper Infected; MP/H=Maris Piper Healthy; G=Guard, cv. Edzell Blue).

	G		G		G			
G	I/NS 7	G	H 8	G	I/S 9	G	**Block 3**	
	G		G		G			
G	H 6	G	I/NS 5	G	I/S 4	G	**Block 2**	
	G		G		G			
G	I/S 1	G	H 2	G	I/NS 3	G	**Block 1**	
	G		G		G			

Figure 3-2. Experiment 2 Randomised block design layout of seed transmission experiment for cv Cara. (I/S=Infected with spraing symptoms; I/NS=Infected with no spraing symptoms; H=Healthy; G=Guard, cv. Edzell Blue).

	Block 4				**Block 3**			
	G		G		G		G	
G	H 8	G	I 7	G	H 6	G	I 5	G
	G		G		G		G	
G	I 1	G	H 2	G	H 3	G	I 4	G
	G		G		G		G	
	Block 1				**Block 2**			

Figure 3-3. Experiment 3 Randomised block design layout of seed transmission experiment for a crop of cv Slaney. (I=Infected; H=Healthy; G=Guard, cv. Edzell Blue).

3.2.2 Seed Transmission Experiment 2005

3.2.2.1 Sample selection and experimental design

Transmission of PMTV infection from seed tubers to daughter tubers was studied in cvs Cara, Nicola, Rooster and Winston. Seed potatoes from two crops of cv. Cara which were identified as having a high incidence of PMTV infection (35% and 52%) were included in this study. As with the 2004 experiments, this trial was laid out in a randomised block design with 3 replications; each plot consisted of 4 drills, each of 10 tubers. Tubers were planted on 10 May, 2005 using the layout shown in Figure 3-4. The crop was monitored during the growing season as described in Sections 3.2.1.1 and 3.2.1.2. The haulm was destroyed on 18 August, 2005 by applying Reglone® at half the rate recommended by the manufacturer, followed by a second application one week later. Daughter tubers were harvested in October, 2005 and tested as previously described in Section 2.2.4.

	G		G		
G	29 C1/I	G	30 N/H	G	
	G		G		
G	28 N/I	G	27 R/I	G	
	G		G		
G	25 C1/H	G	26 C2/I	G	**Block 3**
	G		G		
G	24 W/I	G	23 R/H	G	
	G		G		
G	21 W/H	G	22 C2/H	G	
	G		G		
G	20 W/H	G	19 N/H	G	
	G		G		
G	17 R/I	G	18 C1/H	G	
	G		G		
G	16 C2/I	G	15 N/I	G	**Block 2**
	G		G		
G	13 C1/I	G	14 W/I	G	
	G		G		
G	12 R/H	G	11 C2/H	G	
	G		G		
G	9 W/I	G	10 R/H	G	
	G		G		
G	8 C1/H	G	7 C2/H	G	
	G		G		
G	5 N/I	G	6 C1/I	G	**Block 1**
	G		G		
G	4 W/H	G	3 N/H	G	
	G		G		
G	1 R/I	G	2 C2/I	G	
	G		G		

Figure 3-4. Randomised block design layout of 2005 seed transmission experiment for cvs Cara, Nicola, Rooster and Winston. (C1/H=Cara (crop 1) Healthy; C1/I=Cara (crop 1) Infected; C2/H=Cara (crop 2) Healthy; C2/I=Cara (crop 2) Infected; N/H=Nicola Healthy; N/I=Nicola Infected; R/I=Rooster Infected; R/H=Rooster Healthy; W/H=Winston Healthy; W/I=Winston Infected; G=Guard, cv. Edzell Blue).

3.2.4 Statistical analysis

The variables recorded in the randomised block seed transmission experiments were analysed using logistic regression, as described in Section 2.2.5. Where more than one stock of a single cultivar was included in the experiment (e.g. cvs Nicola and Cara), differences between the means of the main effects and interactions of the treatments were identified by the least significant difference (LSD). LSDs were compared to ensure there were no differences between these stocks. Chi-squared (χ^2) analysis was used on occasion to determine the differences between the interactions and effects of treatments. A two-way general analysis of variance (ANOVA) was used to test the hypotheses concerning the main effects and interaction of treatments on the yield of daughter tubers.

3.3 Results

3.3.1 Seed Transmission Experiments - Health of seed at a common site

A series of four experiments were conducted over 2 years to determine the extent to which plants and daughter tubers produced from PMTV-infected seed tubers became infected and developed disease symptoms. The main criterion for infection was the presence of PMTV in tubers, as determined by ELISA. In addition, in one experiment in 2004, the effect of spraing in PMTV-infected seed tubers on the extent of PMTV transmission was also examined using seed potatoes from an infected crop of cv. Cara.

3.3.1.1 Seed transmission experiments 2004

Symptoms of PMTV infection were observed on some plants of all cultivars derived from infected seed tubers (Tables 3-1, 3-2, 3-4 and 3-5). However, the frequency and type of symptoms which developed on the growing plant differed amongst the cultivars. Symptomatic plants were more frequent with cvs Slaney and Cara than with other cultivars; symptoms of distortion of leaf shape and/or reduction in size of leaflet and stem were mild and largely confined to one or two stems on a plant (Figures 3-5 - 3-12). No plants developed the symptom of mop-top on all stems.

3.3.1.2 Experiment 1

The transmission of PMTV from infected seed tubers to daughter tubers in cvs Maris Piper and Nicola (2 crops) was examined in this experiment. PMTV and its symptoms occurred very rarely (less than 1%) on plants or daughter tubers derived from PMTV-free seed tubers; therefore, comparisons amongst cultivars and seed sources were conducted using PMTV-infected seed only.

Plants with foliar symptoms of PMTV were significantly ($P<0.001$) more frequent with cv. Nicola (23% crop 1; 34% crop 2) than with cv. Maris Piper (4%) (Table 3-1). The incidence of symptomatic plants of cv. Nicola was not significantly affected by seed source although the difference between the two sources was relatively large (23% for crop 1 and 34% for crop 2 (Table 3-2)). With both cultivars, plants developing foliar symptoms of PMTV infection were affected by a reduction in the length of some stems,

leading to stunted growth (Figures 3-5-3-7). Foliage of cv. Nicola also developed chlorotic markings on the leaflets and a distortion of leaf shape; in some cases, these symptoms were also accompanied by stunting (Figures 3-7-3-11).

Table 3-1. Mean % of plants with foliar symptoms of PMTV infection, % plants in which PMTV was detected in leaves and the % daughter tubers infected by PMTV and with spraing symptoms in relation to health of seed tuber (PMTV-free or PMTV-infected) and cultivar (Experiment 1).

PMTV health of seed tuber	Cultivar	% plants with foliar symptoms	% plants PMTV detected	% daughter tubers infected by PMTV	% daughter tubers with spraing
PMTV-free	Maris Piper	0	0	0	0
	Nicola (crop 1)	2	0	1	0
	Nicola (crop 2)	4	2	0	0
Infected	Maris Piper	4	33	34	0
	Nicola (crop 1)	23	12	33	2
	Nicola (crop 2)	34	20	18	1
***P*† (infected seed only)**		**<0.001**	**0.014**	**<0.001**	**<0.001**

†Probability derived from regression analysis on infected seed only.

Table 3-2. Mean % plants with foliar symptoms grown from PMTV-free or PMTV-infected seed of two cultivars.

	Mean % symptomatic plants		
Cultivar	**Infected seed**	**PMTV-free seed**	**LSD (P=0.05)**
Nicola (crop 1)	23.1	2	14.08%
Nicola (crop 2)	34.1	4.4	9.49%
Maris Piper	4.2	0	12.64%

Figure 3-5. Plants of cv. Maris Piper displaying symptoms of PMTV infection. The symptomatic plants are in the foreground, stem length has been reduced leading to a stunted plant. Healthy, normal sized plants of cv. Maris Piper are in the background.

Figure 3-6. A plant of cv. Nicola affected by PMTV. The plant is stunted and is also exhibiting a distortion of the leaflets on one stem.

Figure 3-7. A plant of cv. Nicola displaying symptoms of PMTV infection. The plant is stunted and chlorotic markings are present on the deformed leaflets. Areas of necrosis are also visible on some leaflets.

Figure 3-8. Chlorotic markings and areas of necrosis on the leaves of an affected plant of cv. Nicola.

Figure 3-9. An affected stem on a plant of cv. Nicola with chlorotic markings, areas of necrosis and slight distortion of the leaflets.

Figure 3-10. Symptoms of PMTV infection on one stem of a plant of cv. Maris Piper. Leaflets are misshapen and reduced in size.

Figure 3-11. Chlorotic markings and areas of necrosis on distorted leaflets of cv. Nicola.

During the growing season, leaves of the growing plants were sampled and tested for PMTV by ELISA, as described in Section 3.2.3 and 2.2.4. Virtually all plants from PMTV-free mother tubers were negative for PMTV, although PMTV was found in one plant derived from a PMTV-free seed tuber of cv. Nicola (crop 2). The highest incidence of PMTV in growing plants derived from infected seed tubers occurred in cv. Maris Piper (33%), in which foliar symptoms of this disease were rare. The incidence of PMTV-detection in plants derived from infected seed tubers of cv. Nicola was 12% (crop 1) and 20% (crop 2); but this difference was not significant (LSD (P<0.05)).

The incidence of PMTV infection in daughter tubers of PMTV-infected seed is shown in Tables 3-1 and 3-3. The highest incidence of PMTV infection was detected in the daughter tubers of cv. Maris Piper (34%), closely followed by cv. Nicola (crop 1) at 33%. With cv. Maris Piper, the incidence of infected daughter tubers (34%) was considerably greater than the incidence of plants with foliar symptoms (4%). The incidence of PMTV in daughter tubers in relation to the presence of foliar symptoms on the growing plant is presented in Table 3-3. Overall, the incidence of PMTV in daughter tubers of plants with no foliar symptoms was similar to that for symptomatic plants. The incidence of PMTV in daughter tubers from PMTV-free seed was generally nil or very low, indicating that the tuber test was effective in detecting PMTV and infection from soil inoculum at the trial site was minimal.

Table 3-3. Percentage of PMTV-infected daughter tubers produced from PMTV-infected seed of two cultivars in relation to foliar symptoms on the growing plant.

	% daughter tubers infected with PMTV	
Cultivar	**No foliar symptoms**	**Foliar symptoms**
Maris Piper	33	24
Nicola, crop 1	25	52
Nicola, crop 2	14	14

Spraing was not found in daughter tubers produced from PMTV-free seed tubers nor from PMTV-infected seed tubers of cv. Maris Piper (Table 3-1). Spraing symptoms were observed in daughter tubers derived from infected seed tubers of cv. Nicola, albeit

at very low levels; cv. Nicola (crop 1) (2%) and cv. Nicola (crop 2) (1%). Overall, there was no correlation between spraing symptoms and PMTV infection. For PMTV-infected seed, the incidence of PMTV infection in daughter tubers was much greater than the incidence of spraing symptoms with both cultivars.

At harvest, all daughter tubers were weighed and tuber yield per plant calculated on the basis of the number of plants harvested (Figure 3-12). No significant differences in yield were observed between cvs Maris Piper and Nicola. A two-way general ANOVA showed that the PMTV health of the seed tubers did not affect tuber yield of cv. Maris Piper (P = 0.6) or cv. Nicola (P = 0.2).

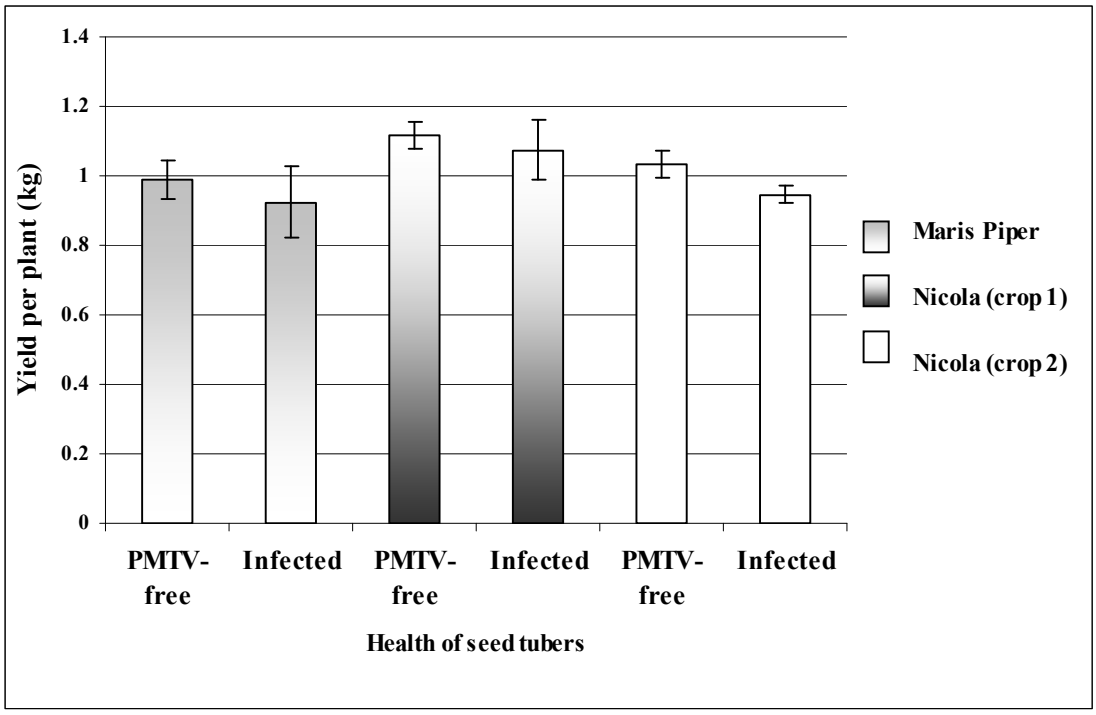

Figure 3-12. Tuber yield with standard error of plants of cv. Maris Piper (d.f.) = 2) and cv. Nicola (two sources of seed potatoes) (d.f.) = 6) in relation to PMTV health of the seed tubers.

3.3.1.3 Experiment 2

As described in Section 3.2.1.1.2, the second experiment in 2004 was conducted to investigate the degree of transmission of PMTV infection from PMTV-infected seed tubers to daughter tubers of cv. Cara and whether this was influenced by the presence of spraing. There were 3 types of seed tubers planted in this experiment: PMTV-free, PMTV-infected with spraing and PMTV-infected with no spraing.

Table 3-4. Mean % plants with PMTV foliar symptoms, % plants in which PMTV was detected in leaves, % daughter tubers infected by PMTV and % daughter tubers with spraing as affected by PMTV health of seed tubers.

		PMTV-infected seed		
	PMTV-free seed	**Spraing**	**No spraing**	***P* value †**
% plants with foliar symptoms	0	30	44	**0.078**
% plants PMTV detected	0	31	18	**0.749**
% daughter tubers infected by PMTV	3	45	54	**<0.001**
% daughter tubers with spraing	0	12	3	**<0.001**

†Probability derived from regression analysis on infected seed only. Differences were considered significant at the $P<0.05$ level

No symptoms of PMTV infection were observed on plants or in daughter tubers derived from PMTV-free seed tubers (Table 3-4). Similarly, PMTV was not detected in leaves of any plants produced from PMTV-free tubers and in only a small quantity (3%) of daughter tubers. The proportion of plants in which PMTV was detected was greater for those from spraing affected tubers (31%) than for those from tubers without spraing (18%) but this difference was not significant. In contrast, the proportion of daughter tubers infected by PMTV was not affected by the presence of spraing in seed tubers for either plants with symptoms ($\chi^2_{(1)} = 0.1$) or plants with no symptoms ($\chi^2_{(1)} = 0.3$), therefore only the mean results for two seed treatments are presented in Table 3-4. The mean incidence of daughter tubers infected by PMTV was significantly greater for those derived from infected tubers with no spraing than for those from spraing affected tubers. Spraing-affected seed tubers also produced a significantly higher incidence of daughter tubers with spraing symptoms than those derived from infected seed tubers in which spraing was absent.

Unlike cvs Maris Piper and Nicola in experiment 1, the yield of daughter tubers of cv. Cara was less for PMTV-infected seed than for PMTV-free seed (P = 0.045) (Figure 3-13). However, the presence of spraing symptoms in infected seed tubers did not affect the yield of daughter tubers (P = 0.215).

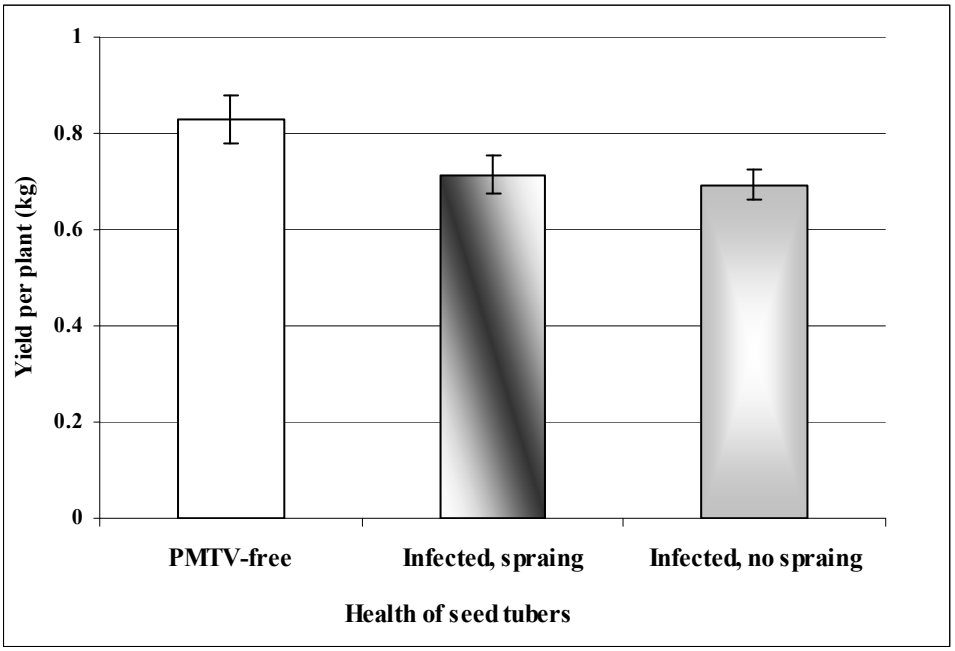

Figure 3-13. Mean tuber yield (with standard error, degrees of freedom (d.f.) = 8) of plants of cv. Cara in relation to health (PMTV-free and PMTV-infected) and symptoms in seed tubers.

3.3.1.4 Experiment 3

The third experiment in the 2004 field experiments investigated the transmission of PMTV from infected seed tubers of cv. Slaney to daughter tubers. Foliar symptoms of PMTV infection were more frequent on plants of cv. Slaney than cultivars in the other two experiments. Approximately 57% of plants grown from infected seed tubers developed symptoms of PMTV infection and a small number of plants from PMTV-free seed also developed symptoms (1.2%) (Table 3-5). PMTV was detected in all of the symptomatic plants grown from PMTV-free seed tubers when the leaves were tested by ELISA as described in Section 2.2.4; however, as with the other cultivars in the trial (with the exception of cv. Maris Piper), the rate of detection of PMTV in foliage of plants derived from infected seed tubers was not much less than the incidence of foliar symptoms (P = 0.34) (Table 3-5). For example, almost 60% of plants of cv. Slaney grown from infected seed tubers showed symptoms of PMTV infection; however PMTV was detected in leaves of only 21%.

Table 3-5. Mean % of plants of cv. Slaney with foliage symptoms, plants in which PMTV was detected and daughter tubers infected by PMTV and with tuber symptoms as affected by health of seed tuber (PMTV-free and PMTV – infected).

PMTV health of seed tuber	% plants with foliar symptoms	% plants infected by PMTV	% daughter tubers infected by PMTV	% daughter tubers with spraing
PMTV-free	1	1	1	0.2
Infected	57	21	42	5
P	**<0.001**	**<0.001**	**<0.001**	**<0.001**

PMTV was detected in a small number of daughter tubers derived from PMTV-free seed tubers. Cultivar Slaney was the only cultivar in the 2004 experiments in which spraing symptoms developed in daughter tubers derived from PMTV-free seed tubers (0.2%). The incidence of spraing symptoms in daughter tubers produced from infected seed tubers was also relatively low (5%) but significantly greater from that for PMTV-free seed tubers. The yield of daughter tubers was not affected by the health of the seed tubers ($P = 0.14$) (Figure 3-14).

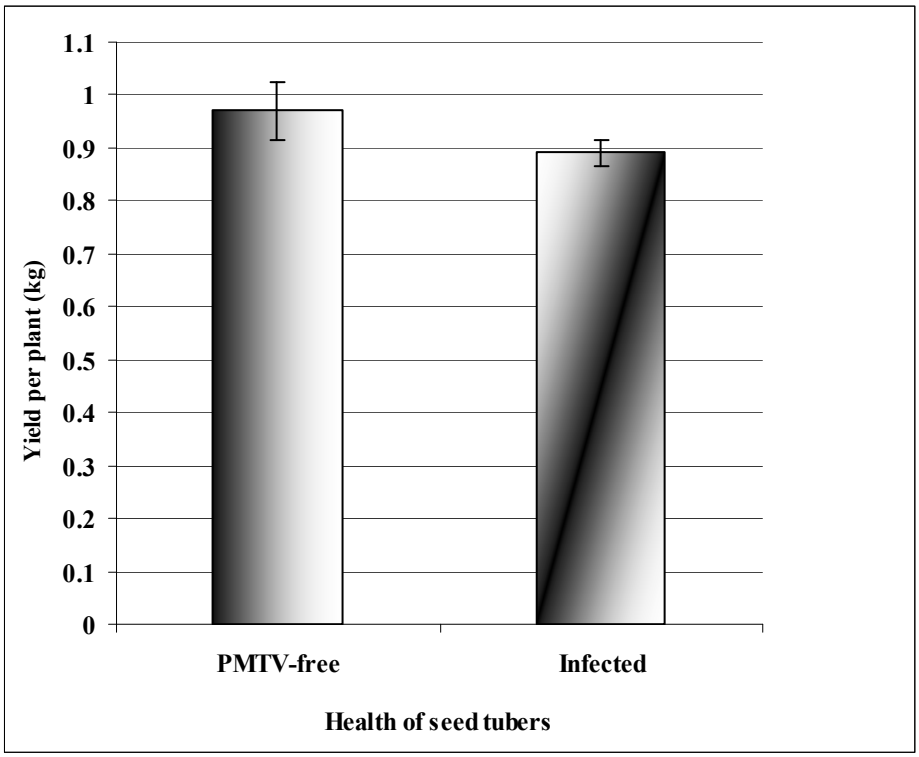

Figure 3-14. Mean tuber yield (kg) (with standard error (d.f.) = 7) of plants of cv. Slaney as affected by the health of seed tuber (PMTV-free and PMTV-infected).

3.3.2 Seed transmission experiment 2005

In 2005, the transmission of PMTV was studied in four cultivars as described in Section 3.2.2. Foliar symptoms of PMTV infection on plants grown from PMTV-free seed were rare (Table 3-6). Of all the plants produced from PMTV-free seed tubers, only one (cv. Cara (crop 2)) developed foliar symptoms characteristic of PMTV infection. With infected seed tubers, symptoms were most common on plants of cv. Nicola (15%) and none developed on plants of cvs Rooster and Winston. However, the incidence of plants of cv. Cara with foliar symptoms was less in 2005 than in 2004 (mean of 10% in 2005 compared with a mean of 37% in 2004).

As with the 2004 results, statistical analysis was performed only on data for the infected seed tuber treatment because the values for the PMTV-free seed tuber treatment were zero or close to zero.

Table 3-6. Mean % of plants with foliar symptoms of PMTV infection, % plants in which PMTV was detected in leaves and the % daughter tubers infected with PMTV and with spraing symptoms as affected by health of seed tuber and cultivar.

PMTV health of seed tuber	Cultivar	% plants with foliar symptoms	% plants in which PMTV detected	% daughter tubers infected by PMTV	% daughter tubers with spraing
Healthy	Cara (crop 1)	0	0	1	0
	Cara (crop 2)	0.8	0	0.2	0.2
	Nicola	0	2	0.9	0
	Rooster	0	0	0.2	0
	Winston	0	0	0.2	0
Infected	Cara (crop 1)	12.5	32	34	10.5
	Cara (crop 2)	9.2	36	41	5.9
	Nicola	15	30	32	1.1
	Rooster	0	27	23	1.3
	Winston	0	40	38	0.2
***P* † (infected seed only)**		**< 0.001**	**0.48**	**< 0.001**	**< 0.001**

† Probability derived from regression analysis on infected seed only

Virtually all plants derived from PMTV-free seed tubers tested negative for PMTV by ELISA except for one asymptomatic plant of cv. Nicola. However, PMTV was not detected in a symptomatic plant of cv. Cara (crop 2) grown from PMTV-free seed. With PMTV-infected seed, the incidence of plants in which PMTV was detected did not differ significantly with cultivar (Table 3-6) even though there were significant differences amongst cultivars in foliar symptom development. With all cultivars, the incidence of detection of PMTV in asymptomatic plants derived from PMTV-infected seed was relatively high (21-40%) (Table 3-7). With cvs Cara and Nicola, however,

PMTV was detected twice as frequently in symptomatic plants than in asymptomatic plants (mean detection of 53% and 26% respectively) (Table 3-7).

Table 3-7. The incidence of detection of PMTV in leaves of plants of four cultivars derived from PMTV-free or PMTV-infected seed tubers in relation to presence of foliar symptoms on the growing plant.

PMTV health of seed tuber	Cultivar	No foliar symptoms	Foliar symptoms
PMTV-free	Cara (crop 1)	0 (48)*	-†
	Cara (crop 2)	0 (48)	0 (1)
	Nicola	1 (48)	-†
	Rooster	0 (48)	-†
	Winston	0 (48)	-†
Infected	Cara (crop 1)	29 (48)	40 (15)
	Cara (crop 2)	29 (48)	64 (11)
	Nicola	21 (48)	56 (18)
	Rooster	27 (48)	-†
	Winston	40 (48)	-†

† Symptomatic plants not observed
* Total number of plants tested is shown in parentheses

Although PMTV was detected in daughter tubers produced from PMTV-free seed of all cultivars, the amount of infection was very low (Table 3-6). The incidence of PMTV infection in daughter tubers derived from PMTV-infected seed tubers ranged from 23% for cv. Rooster to 41% for cv. Cara (2).

The incidence of PMTV infection in daughter tubers derived from PMTV-infected seed tubers was slightly greater for plants with foliar symptoms (40%) than for plants with no symptoms (35%) in 2 out of 3 comparisons (Table 3-8). However, the results could

not be analysed statistically as the absence of symptomatic plants in some cultivars skewed the results towards infected tubers derived from asymptomatic plants and therefore would have compromised the accuracy of any potential statistical analysis. The highest incidence of PMTV in daughter tubers of asymptomatic plants derived from infected seed was 40% for cv. Cara (2), which was identical to that for symptomatic plants.

Table 3-8. Percentage of PMTV-infected daughter tubers produced from PMTV-infected seed in relation to foliar symptoms and cultivar.

	Mean % tubers infected by PMTV	
Cultivar	**Foliar symptoms**	**No foliar symptoms**
Cara (crop 1)	41 (221)†	34 (450)
Cara (crop 2)	40 (183)	40 (450)
Nicola	40 (212)	31 (450)
Rooster	NA	23 (450)
Winston	NA	38 (450)

†Figure in parenthesis is the number of tubers tested.

Spraing was found in daughter tubers derived from PMTV-free seed in only one cultivar, and in a very low number (Table 3-6). With infected seed tubers, the highest incidence of spraing occurred in daughter tubers of cv. Cara, although spraing was more common for Cara (crop 1) than Cara (crop 2), which was the reverse of the incidence of tuber infection. The frequency of occurrence of spraing in the other three cultivars was less than 1.5%.

Figure 3-15 presents the yield per plant for each cultivar in relation to the health of the seed tubers. With the exception of cv. Winston, plants from infected seed tubers produced a significantly ($P < 0.001$) smaller yield than those from PMTV-free seed tubers.

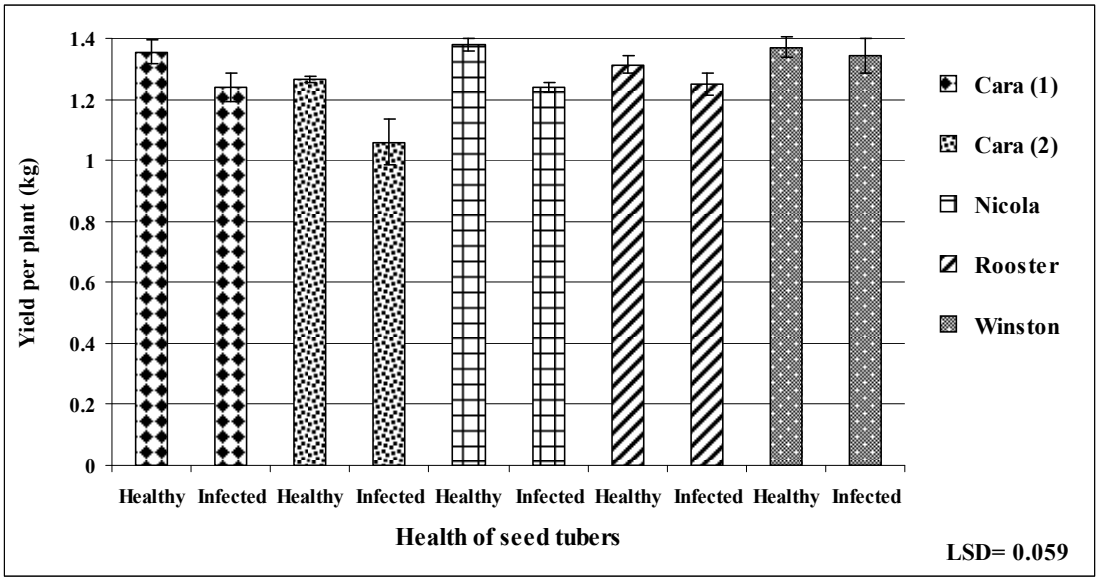

Figure 3-15. Mean tuber yield (kg) of plants of cvs Cara, Nicola, Rooster and Winston (with standard error bars (d.f.) = 29), as affected by health of seed tubers (PMTV-free or PMTV-infected).

3.4 Discussion

3.4.1 The importance of seed transmission in PMTV infection

The aim of these experiments was to determine the efficiency of PMTV transmission from an infected seed tuber to the growing plant and to daughter tubers in a range of commonly grown cultivars. The experiments were conducted at a common site, known to be PMTV-free, over two years.

3.4.1.1 Symptoms in the growing plant

In the 2004 and 2005 seed transmission experiments, foliar symptoms on most varieties were relatively mild, usually consisting of distortion or discolouration of leaflets on one or two stems, accompanied by some reduction in length of the affected stem. No plants developed the mop-top symptoms of dwarfing and bunching of leaves affecting all stems. This may be attributed to either the prevailing environmental conditions during the growing seasons, or because the cultivars included in this study are not sensitive to PMTV foliar infection. Calvert (1968) reported anecdotal evidence that mop-top symptoms developed in response to chilling early in the season. In this study, with the exception of cv. Slaney, the frequency of PMTV foliar symptoms in plants derived from PMTV-infected tubers, did not exceed 50%. This finding with PMTV-tested tubers is similar to those of other studies using symptomatic tubers (Calvert, 1968; Cooper and Harrison, 1973; Salazaar and Jones, 1975). Calvert (1968) planted spraing-affected tubers of 29 cultivars; foliar symptoms developed on plants of all cultivars, however, the frequency differed amongst cultivars. Fourteen percent (4/29) of the cultivars studied had more than 50% of plants expressing foliar symptoms of PMTV infection. The highest incidence of symptomatic plants was observed in cv. Ulster Ranger (79%) and the lowest in cv. Dunbar Standard (4%). However, fewer tubers of cv. Dunbar Standard were planted compared to cv. Ulster Ranger which may have skewed the results. Observations on growing crops by Cooper and Harrison (1973) and Salazaar and Jones (1975) found that the incidence of plants with foliar symptoms did not exceed 48% and 40% respectively.

The development of foliar symptoms on plants derived from PMTV-infected seed was found to be cultivar dependant. In 2004, symptoms were more prevalent on cvs Slaney (57%) and Cara (30 and 44%) than on cv. Maris Piper, on which only 4% of plants were

symptomatic. In the 2005 experiment, foliar symptoms occurred much less frequently overall, being most frequent on cv. Nicola, albeit at a comparatively low level (15%) with no symptoms observed in cvs Rooster and Winston. A more recent study by Carnegie *et al.* (2009) also found a higher frequency of symptoms in plants of cv. Slaney derived from PMTV-infected seed tubers, relative to most of the other cultivars studied. It would, therefore, appear that foliage of cv. Slaney is particularly sensitive to infection by PMTV. In the Carnegie *et al.* (2009) study, in agreement with my results, no symptoms were observed on plants of cv. Rooster. However, some symptoms were observed on plants of cv. Winston, in contrast to the present study. Conversely, in the present study, symptoms of infection were observed in plants of cv. Maris Piper, albeit at low levels but were not found by Carnegie *et al.* (2009).

Foliar symptoms caused by PMTV develop only on plants produced by infected seed tubers and not as a result of primary infection, which is infection which occurs from soil-borne inoculum within the current growing season (Calvert and Harrison, 1966; Harrison and Jones, 1970; Kurppa, 1989a). Harrison and Jones (1970) reported that 62% of plants produced from spraing-affected tubers (obtained by dusting the seed tubers with PMTV-carrying *S. subterranea* sporeballs) of cv. Arran Pilot displayed symptoms of PMTV infection, with no symptoms observed in control plants which were derived from tubers of cv. Arran Pilot which had not been exposed to sporeballs. Cooper *et al*., (1976) also demonstrated the relationship between tuber infection and foliar symptom expression in plants by growing on tubers from plants of cv. Red Craig's Royal with or without foliar symptoms of PMTV infection. Their findings indicate that planting tubers from plants with no symptoms does not result in plants with foliar symptoms; however, due to a very low number (4) of samples in this study, combined with a lack of replication, these findings cannot be considered conclusive.

In contrast to these earlier findings, the results presented here show that foliar symptoms were observed on a low number of plants derived from tested PMTV-free tubers of three cultivars; Nicola (4% and 2%), Cara (0.8%) and Slaney (1%). The source of PMTV causing these symptoms on plants grown from tubers of these cultivars is probably missed detection in the tuber testing; Sokmen *et al.* (1998) also reported that 3 out of 12 apparently PMTV-free tubers produced infected daughter plants, which they considered may have arisen because of discrepancies in sampling the tuber flesh. As foliar symptoms are not generally associated with primary infection (Calvert, 1968;

Harrison and Jones, 1970), it is possible that a small number of seed tubers were infected by PMTV but the virus titre was below the level of detection by ELISA at the time of testing, or that PMTV was not detected in infected tubers because the virus was discontinuously distributed in the tubers, as found previously by Torrance *et al.* (1992).

A higher frequency of plants with foliar symptoms was observed in the 2004 experiments than in the 2005 experiment. Two cultivars (Cara and Nicola) were included in both years and both cultivars had fewer affected plants in 2005. Jones and Harrison (1972) also observed differences in the incidence of foliar symptomatic plants of cv. Red Craig's Royal between the two years of their study on commercial seed crops. These differences were thought to be attributable to variation in environmental factors between years. Certainly, Harrison and Jones (1970) stated that symptom development on inoculated leaves and subsequent systemic infection by PMTV in a range of host plants, such as, *Nicotiana debneyi* and *Chenopodium amaranticolor*, was favoured by cool conditions or sudden changes in temperature; however, no experimental evidence currently demonstrating the relationship between environmental factors and foliar symptom expression of PMTV infection in potato exists.

In the 2004 study, foliar symptoms of PMTV infection were observed on a greater proportion of plants of cv. Cara derived from PMTV-infected tubers without spraing symptoms, than those derived from PMTV-infected tubers with spraing symptoms. In contrast, Jones and Harrison (1969) reported that foliar symptoms were significantly more frequent (67%) on plants of cv. Arran Pilot produced by spraing-affected seed tubers than those without spraing (53%). It is, however, impossible to draw conclusions on the basis of results obtained from two cultivars and further study is required to determine whether spraing influences PMTV transmission from mother to daughter tuber.

While the results presented here provide an insight into varietal differences with regard to the extent of foliar symptom expression in plants derived from PMTV-infected seed tubers, these studies did not quantitatively record the specific types of symptoms observed on plants of, and within, the various cultivars. Future studies may, therefore, need to record the nature and incidence of symptoms although these may well be subject to environmental influences. It is, however, evident from the results presented here, and previous studies, that symptoms of PMTV infection in the growing plant are not a

reliable indicator of the actual amount of PMTV infection present in a crop, even when a variety is sensitive.

3.4.1.2 Detection of PMTV in leaves in relation to the presence of foliar symptoms in the growing plant

The relationship between the occurrence of foliar symptoms and detection of PMTV in the growing plant was investigated. Three of the five crops exhibited symptoms of PMTV; two crops of cv. Cara and one of cv. Nicola at 12.5%, 9.2% and 15%, respectively. For both cultivars, PMTV was detected in a greater number of plants with foliar symptoms than without. However, PMTV was not detected in many symptomatic plants (36-60%) although symptomatic leaves were tested. This suggests that the distribution and concentration of virus may vary within the plant. Montero-Astúa *et al*., (2008) also found a poor correlation between foliar symptoms and infection in the plant, as detected by ELISA, with only 28% of symptomatic plants and 11% of asymptomatic plants testing positive for PMTV. It is probable that some of the infections in asymptomatic plants may have been the result of infection from soil inoculum.

In all cultivars studied here, PMTV was detected in a proportion of plants with no foliar symptoms produced from infected seed tubers. PMTV detection in these asymptomatic plants ranged from 21% in cv. Nicola to 40% in cv. Winston. In all 2005 crops, substantial differences were found between the incidence of foliar symptoms in the growing plant and the extent of infection, as determined by ELISA. These results demonstrate that the infection in a considerable proportion of plants derived from infected seed may be asymptomatic. These findings contradict those of a previous study by Calvert and Harrison, (1966) who did not detect any PMTV in symptomless plants. However, indicator plants were used to detect PMTV infection and it is clear from their study, and from that of Harrison and Jones (1970), that sap inoculation of indicator plants is not a reliable method of PMTV detection. A study by Tenorio *et al.* (2006), in which the effect of PMTV infection was examined in 21 cultivars from the USA, confirms the finding that foliar symptoms are an unreliable indicator of PMTV infection. The results in this study, clearly demonstrate that ELISA testing of leaves in a growing crop provide a more accurate estimation of the actual incidence of PMTV infection but this is likely to underestimate infection as demonstrated by missed detection on plants with foliar symptoms of PMTV.

3.4.2 Transmission of PMTV from infected seed tubers to daughter tubers

The results from these studies show that transmission of PMTV from seed to daughter tubers is relatively inefficient, in comparison with the transmission of other common viruses of potato e.g. leafroll, PVY, with yield losses as high as 100%, if a crop is grown from infected seed tubers (Barker and Woodford, 1987 and Valkonen, 2007). In all cultivars studied here, it was demonstrated that PMTV is only transmitted to a proportion of daughter tubers from infected mother tubers but the efficiency seemed to be broadly the same for sensitive and tolerant cultivars. The most efficient transmission was found in a crop of cv. Cara studied in 2004, in which 49% (composite of infection in both samples with and without spraing) of daughter tubers were infected. The least efficient transmission was found with cv. Rooster, in which only 23% of daughter tubers were infected.

Experiments by Calvert (1968) and Cooper *et al.*, (1976) using infected seed tubers of cvs Arran Pilot and Red Craig's Royal respectively, also confirmed that the virus is transmitted to only a proportion of daughter tubers. However, in their experiments, the transmission of PMTV from one generation to another was assessed on the basis of plants with foliar symptoms and not by the presence of virus in the foliage or tuber, as reported here. Nevertheless, the results of the current study confirm that, in the absence of new infections, the incidence of PMTV infection in a crop will be reduced with each propagation cycle and may lead to the elimination of the virus. Calvert (1968) estimated that the incidence of PMTV infection, as assessed by recording symptomatic plants, would reduce to 3% after 3 years. Assuming the rate at which PMTV self eliminates from a crop remains constant, Calvert's estimate may be accurate for some cultivars but not others; for example, in 2005, cv. Rooster, in which PMTV was detected in 23% of daughter tubers, PMTV infection would be reduced to 1% after three years propagation (assuming 100% infection in year 1). However, for cv. Cara, in which PMTV was detected in 49% of daughter tubers, PMTV infection would only be reduced to 12% in three years, requiring a further three to four years multiplication in order to eliminate the virus from the crop. However, there appeared to be a considerable difference in the efficiency of transmission within cultivars as demonstrated with different seed sources of cv. Nicola in 2004. The results for specific cultivars should therefore be treated with some caution.

Overall, previous studies have collectively relied on symptom expression in either tubers or plants to indicate PMTV infection. As all seed tubers and progeny tubers in this study were tested for PMTV by ELISA, the results presented here fully account for symptomatic and symptomless PMTV tuber infection. These results are therefore a more accurate reflection of the efficiency of transmission of PMTV from infected seed tubers to progeny tubers than results from previous studies.

3.4.3 The effect of PMTV infection on yield of daughter tubers

The effect of PMTV infection on tuber yield was found to be variable. The difference in yield between plants grown from PMTV-infected and PMTV-free seed tubers ranged from a 2% decrease in yield with cv. Winston to a 17% decrease with cv. Cara. In the majority of comparisons in 2004 and 2005, plants from PMTV-infected tubers produced yields which were slightly lower, but not significantly so, from those grown from PMTV-free seed tubers, with the exception of the 2004 crop of cv. Cara where the difference was significant. These findings reflect previously published work, in which the yield of plants from infected seed tubers was sometimes reduced by up to 37% (Kurppa (1989a). In this study, however, significant reductions occurred with only 4 out of 8 comparisons and, as noted above, there was considerable variability in reaction within a cultivar with only one out of three comparisons with cv. Saturna being significant. Kurppa (1989a) indicated that loss of yield was associated primarily with delayed and uneven emergence. In Denmark, Nielsen and Mølgaard (1997) reported that the yield of crops of cv. Saturna with spraing at harvest was similar to that of those without spraing. However, it seems likely that infection occurred in that growing season which might not be expected to have any effect on plant growth and hence yield. By contrast, Calvert (1968) reported that planting tubers with PMTV spraing resulted in a smallerr reduction in yield than planting tubers with secondary symptoms of malformation or cracking. With the sensitive cv. Arran Pilot, reductions of 10% occurred with primary symptoms and of 26% with secondary symptoms. With the foliar tolerant cv. Ulster Premier, however, no reduction in yield was recorded when tubers with spraing were planted, even though 25% of plants developed symptoms compared with 5% for symptomless seed tubers. Similarly, in the 2004 and 2005 experiments reported here, no significant reductions in yield of tolerant cultivars were recorded.

Chapter 4. Common origin seed and soil-borne inoculum

4.1 Introduction

The aim of this part of the study was to establish the relative importance of seed and soil inoculum in causing outbreaks of PMTV in seed potato crops. Irish Potato Marketing Ltd (IPM), who are agents for breeders of cv. Cara, had a unique system of seed production which was appropriate for the study. In order to provide seed potatoes for the UK washed, pre-packed, and Canary Islands and Egypt export markets, IPM authorised one pre-basic producer in Ross and Cromarty to multiply initial seed potatoes for a number of generations. This common origin seed is then distributed to over 25 producers of basic seed potatoes for multiplication over two field generations. In the first year, these producers plant the common origin seed to produce certified farm saved seed which is planted the following year to produce seed potatoes for final marketing (Table 4-1).

Table 4-1. Pathway of seed production of cv. Cara by basic seed producers in Scotland.

Year	Production cycle
0	Common origin seed tubers (1 producer) ↓
1	Certified farm saved seed (>25 producers) ↓
2 +	E1 Seed Potatoes marketed to the UK and other markets

The health of seed and daughter tubers of cv. Cara was studied over 2 series of multiplication cycles between 2004 and 2006. Samples of common origin seed tubers were collected by seed potato inspectors at the producer's farm in Ross and Cromarty. In 2004, the seed potatoes were from 2 crops, one class SE1 and the other class SE2, and in 2005, only seed potatoes from an SE2 crop were distributed to the basic producers.

A further experiment to determine the relative importance of temperature on the transmission of PMTV from infested soil was also studied in this section. These experiments were conducted in separate glasshouses over a range of temperatures. The

study also gave an insight into the effect of temperature on expression of spraing symptoms in infected tubers and the occurrence of powdery scab at a range of temperatures.

4.2 Materials and Methods

4.2.1 Seed transmission experiment methodology

4.2.1.1 Sample selection

Seed tubers of 2 Super Elite (SE1 and SE2) crops of cv. Cara grown on the Black Isle (Ross and Cromarty) in the North of Scotland were tested for PMTV, as were daughter crops over two multiplication seasons. The location of crops, the farm number, the class of seed studied and the grower number, assigned specifically for this study, are presented for crops studied in 2004 (Table 4-2), 2005 (Table 4-3) and 2006 (Table 4-4).

Table 4-2. Details of 31 crops of class Super Elite of cv. Cara studied in 2004. Crops were grown throughout Scotland.

Area/Grower/Farm	Entry grade at classification
Banff/28/1	SE3
Angus/25/1	SE3
Angus/27/1	SE3
Angus/17/1	SE3
Angus/2/1	SE3
Angus/8/1	SE3
Angus/13/1	SE3
Angus/33/1	SE3
Angus/34/1	SE3
Angus/24/1	SE3
Angus/11/1	SE3
Angus/22/1	SE3
Angus/14/1	SE3
Perth/6/1	SE3
Angus/20/1	SE3
Perth/10/1	SE3
Perth/19/1	SE3
Perth/9/1	SE3
Perth/3/1	SE3
Fife/23/1	SE3
Aberdeen/21/1	SE2
Angus/5/1	SE2
Angus/30/2	SE2
Angus/26/1	SE2
Angus/18/1	SE2
Angus/12/1	SE2
Angus/7/1	SE2
Perth15/1	SE2
Perth/31/1	SE2
Fife/16/1	SE2
Moray/4/1	SE2

Table 4-3. Eighty-four seed crops of cv. Cara studied in 2005. The majority of these crops were derived from seed tubers which were included in the 2004 study, whilst the remainder were derived from seed potatoes produced by the initial grower.

Area/grower/ farm	Entry grade at classification	Area/grower/ farm	Entry grade at classification	Area/grower/ farm	Entry grade at classification
Aberdeen/21/1	SE3	Angus/2/2	E1	Angus/12/5	SE3
Angus/27/2	SE3	Angus/2/3	E1	Perth/10/1	E1
Angus/25/2	SE3	Angus/2/3	E1	Moray/4/2	SE3
Banff/28/1	SE3	Aberdeen/21/1	SE3	Moray/4/1	SE3
Perth/3/2	SE3	Angus/13/1	E1	Moray/4/1	SE3
Perth/19/2	SE3	Angus/14/1	E1	Angus/25/3	E1
Angus/22/1	SE3	Angus/14/1	E1	Angus/7/1	SE3
Angus/6/2	SE3	Angus/14/2	E1	Perth/15/1	SE3
Moray/4/1	SE3	Angus/14/2	E1	Roxburgh/32/1	SE3
Perth/9/2	SE3	Angus/5/2	SE3	Angus/22/1	E1
Perth/31/1	SE3	Angus/5/3	SE3	Angus/22/2	E1
Aberdeen/29/1	SE3	Fife/23/3	E1	Perth/6/1	E1
Aberdeen/29/2	SE3	Fife/23/4	E1	Angus/6/2	E1
Angus/13/1	SE3	Perth/19/2	E1	Perth/3/3	E1
Angus/17/1	SE3	Angus/27/2	E1	Perth/3/4	E1
Angus/18/2	SE3	Angus/30/1	SE3	Perth/3/1	E1
Angus/6/2	SE3	Angus/30/1	SE3	Perth/3/1	E1
Angus/30/1	SE3	Angus/18/2	SE3	Perth/20/2	E1
Angus/12/2	SE3	Angus/17/3	E1	Angus/20/3	E1
Angus/11/2	SE3	Angus/17/2	E1	Angus/11/2	E1

Table 4-3 Continued. Eighty-four seed crops of cv. Cara studied in 2005. The majority of these crops were derived from seed tubers which were included in the 2004 study, whilst the remainder were derived from seed potatoes produced by the initial grower.

Area/grower/ farm	Entry grade at classification	Area/grower/ farm	Entry grade at classification	Area/grower/ farm	Entry grade at classification
Angus/7/3	E1	Fife/16/1	SE3	Angus/11/3	E1
Angus/7/2	SE3	Perth/9/5	E1	Banff/28/1	E1
Angus/2/2	SE3	Angus/12/3	SE3		
Angus/14/2	SE3	Angus/12/3	SE3		
Fife/23/2	SE3	Angus/12/3	SE3		
Fife/16/1	SE3	Angus/12/4	SE3		
Perth/10/1	SE3	Angus/12/4	SE3		
Perth31/2	SE3	Angus/12/4	SE3		
Perth/9/2	E1	Angus/12/4	SE3		
Perth/9/4	E1	Angus/12/2	SE3		
Perth/9/3	E1	Angus/12/5	SE3		

Table 4-4. The location and grower reference number of the 28 crops of cv. Cara sampled in 2006.

Area/grower/farm	Entry grade at classification
Fife/23/5	E1
Fife/23/1	E1
Angus/22/2	E1
Angus/22/1	E1
Angus/22/3	E1
Angus/17/1	E1
Angus/17/4	E1
Angus/14/3	E1
Angus//14/4	E1
Angus/5/2	E1
Angus/5/3	E1
Perth/9/6	E1
Perth/9/5	E1
Perth/9/1	E1
Perth/19/3	E1
Perth/19/4	E1
Aberdeen/29/2	E1
Angus/25/2	E1
Perth/31/2	E1
Angus/30/2	E1
Angus/13/1	E1
Angus/27/3	E1
Banff/28/1	E1
Aberdeen/21/1	E1
Angus/7/2	E1
Perth/3/1	E1
Perth/3/2	E1
Angus/11/4	E1

4.2.1.2. Processing tuber samples

Every year after harvest, *c.* 200 tubers from each crop were collected by SGRPID inspectors and dispatched to SASA. Once all samples had been received, tubers were stored in conditions conducive to spraing development (see Section 2.2.2). Samples were washed and visually assessed for the presence of powdery scab lesions. Following the powdery scab assessments, tubers tested for PMTV by ELISA (see Section 2.2.4) and then were sliced and assessed for the presence and severity of spraing symptoms (Figure 2-2). All results are given as an overall percentage for each stock. In 2004, 200 tubers from each farm were tested; however, in 2005 and 2006, testing 200 tubers was impractical because of the increased number of samples and limited resources for testing so 150 tubers from each farm were tested in both these years for PMTV, spraing and powdery scab.

4.2.2. Detection of PMTV in soil

4.2.2.1 Introduction

The purpose of these experiments was to develop a reliable, sensitive, and, more importantly, reproducible test for PMTV in soil, initially by determining the most effective host plant to use with respect to sensitivity using real-time RT-PCR, and the optimal time to harvest the bait plants.

4.2.2.2 Soil Bait Plant Assay

Prior to testing, soil was spread out on 30 x 40cm plastic trays, broken up to remove large lumps, if necessary, and dried in drying cabinets, set at room temperature, for at least one week. Twenty grams of the dried sample soil was placed into a well of a 5 x 9 multicell tray (AMP SpA, Ferrara, Italy). The soil was moistened and a two week old tomato seedling (*Lycopersicum esculentum* cv. Moneymaker) was transplanted into each well. Three replicate wells were used for each soil sample (see Figure 4-1). A positive control (known infested soil from a site near Auchterarder, Perthshire, Scotland) and negative control (John Innes Compost No. 2) were included on each multicell tray. The seedlings were grown under natural light conditions in a glasshouse at temperatures between 17 and 21°C and watered daily. After two weeks, the bait

plants were harvested by carefully removing them from the wells. The roots were rinsed under running tap water and *c.* 100mg of root tissue was excised from each bait plant using a scalpel which was sterilised by soaking sequentially in water, 0.2M NaOH and 96% ethanol (aq., v/v) and flaming. The roots from each bait plant were placed into an individual homogenisation bag prior to extracting total RNA from the tissue.

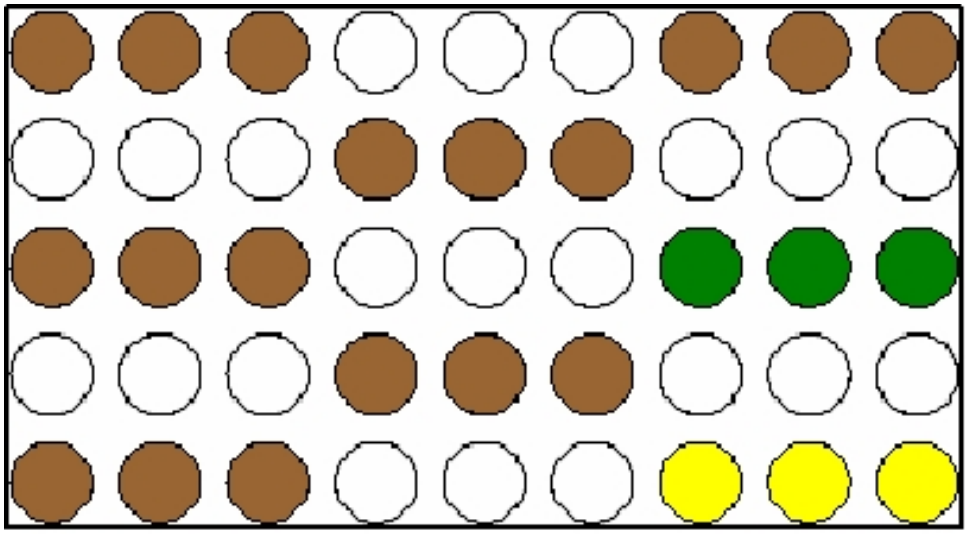

Figure 4-1. Diagram of multicell tray illustrating the bait plant set up. Soils were placed in alternate wells to prevent carryover. A positive (green) and negative (yellow) control soil sample was included on each tray.

4.2.2.3 Total RNA Extraction

Total RNA extractions were carried out using the Kingfisher mL™ system (Thermo Labsystems, Finland) and Magextractor®-RNA-reagents (Toyobo, Japan), according to the manufacturer's instructions, with the exception that 2-mercaptoethanol was not added to the lysis buffer. The extraction method involves the use of magnetic particles for purifying total RNA from tissue homogenates. The RNA is then eluted from the magnetic particles. This method was found to be reliable and time efficient with 15 samples being processed in approximately 30 minutes.

Root tissue was ground in the homogenisation bag in the presence of 1mL of lysis buffer (Toyobo, Japan) using a Homex 5 homogeniser (Bioreba AG, Switzerland). Kingfisher mL tube strips were prepared with Magextractor®-RNA-reagents (Toyobo, Japan). Each sample required one strip (Figure 4-2). One millilitre of the tissue homogenate and 50µl magnetic particles were added to well 1 of the tube strip. Well 2

contained 1ml RNA Washing Buffer 1. Into wells 3 and 4, 1ml RNA Washing Buffer 2 was pipetted. Finally, 200µl RNA Elution buffer was added to well 5.

The homogenised tissue was incubated with magnetic particles for 19 minutes. Following incubation, the particles were washed in RNA Washing Buffer 1 (well 2). Washing of the magnetic particles was then repeated with RNA Washing Buffer 2 in wells 3 and 4. The particles were then released in RNA Elution Buffer in well 5. During the elution, the samples were removed from the Kingfisher mL™ system and incubated at 65°C for 5 minutes. The samples were then returned to the Kingfisher mL™ system and the particles removed from well 5 and transferred to well 4. Extracted RNA template was collected from well 5 and stored at -20°C prior to testing by real-time RT-PCR.

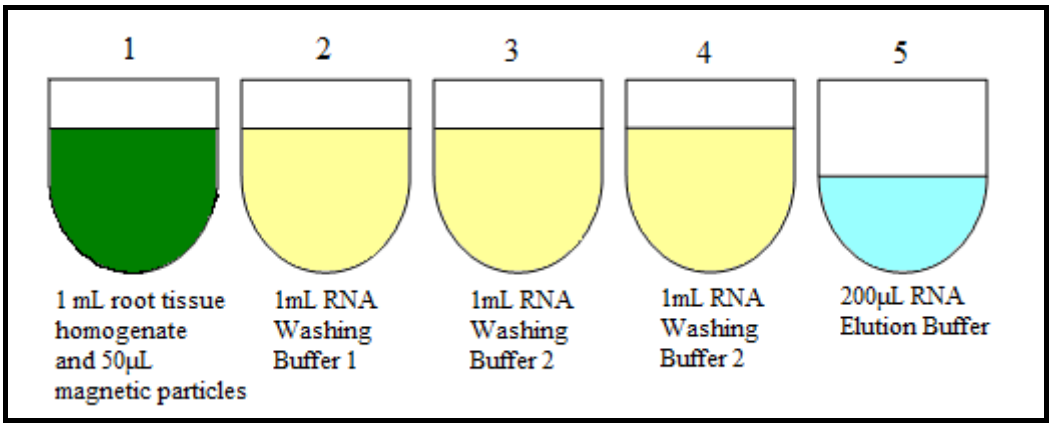

Figure 4-2. Side profile of Kingfisher mL tube strip containing Magextractor®-RNA-reagents.

4.2.2.4. Real-time Reverse Transcriptase-PCR (RT-PCR) for detection of PMTV

4.2.2.4.1 Primers and probes

Probes and primer pairs were designed using Primer Express™ Version 1.5 (Applied Biosystems, U.K) and synthesised by MWG-Biotech AG (Ebersberg, Germany). Two primer pairs and a separate probe were designed for each RNA molecule in the PMTV genome. Tables 4-5 and 4-6 show the nucleotide sequences for the primers and probes. The fluorogenic probes were labelled at the 5′ end with the fluorescent reporter dye 6-FAM® (6-carboxy-fluorescein) and modified at the 3′ end with the quencher dye TAMRA® (6-carboxy-tetramethylrhodamine). The effectiveness of these primer-probe combinations was also compared with that of one set of primers designed by Mumford *et al.* (2000) (Table 4-7) which targets the RNA 3 molecule.

Table 4-5. Nucleotide sequences of primers used for the amplification of the three RNA molecules in the PMTV genome. Two primer combinations were designed for each RNA molecule[5].

Primer Name	Nucleotide sequence (5′ to 3′)
RNA1A_F	AGGACAGCTATGCCCGAGAA
RNA1A_R	GGTGCAGCCATATTTCGCTT
RNA1B_F	TGGGTCGTGCATGGACCTA
RNA1B_R	GACCGAAGTCTTGTAAGCACTAACAT
RNA2A_F	CCGACATAAGTTTGY*GCTTG
RNA2A_R	TCGATGY*CAATTCTCCGTTAA
RNA2B_F	AGAATTGR*CATCGAAACAGCA
RNA2B_R	GTCGCGCTCCAATTTCGTT
RNA3A_F	GGTTACGCTGGGCTGGTG
RNA3A_R	CGATAGCGGCGTTGACG

[5] *, Y encodes any pyrimidine base, i.e. C, T or U; R encodes any purine base, i.e. A or G

Table 4-6. Nucleotide sequences of probes used for the amplification of the three primer combinations which were designed for each RNA molecule.

Probe name	Nucleotide sequence (5´ to 3´)
RNA1A_P	AGCGGTTTGGCAGAAGGTTTGTTGG
RNA1B_P	TCAAAACATGCCGTGGACATTACGTCA
RNA2A_P	AATCCGTTGTAATCCAGAACTGTTCATGCAG
RNA2B_P	CCACAAACAGACAGGTATGGTCCGGAA
RNA3A_P	AGCAATTAACCGCTCAGGCTTTTTGGTTTG

Table 4-7. Nucleotide sequences of the primers and probe combination designed by Mumford *et al.* (2000).

Name	Nucleotide sequence (5´ to 3´)
RNA3B_F	GTGATCAGATCCGCGTCCTT
RNA3B_R	CCACTGCAAAAGAACCGATTTC
RNA3B_P	ACCAGAACTACGGTGCCGCGTCG

4.2.2.4.2 Real-time RT-PCR Amplification

Real-time RT-PCR was performed on 1µL of RNA template in 25µL reaction mixtures. A standard 25µL reaction mixture for the amplification of PMTV RNA sequences is shown below in Table 4-8.

Table 4-8. Reagents required for a standard 25µL real-time RT-PCR reaction mixture.

Real-time RT-PCR component	Amount (µL)
JumpStart™TaqReadyMix for Quantitative PCR*	12.5
$MgCl_2$ (3mM)	4.0
PMTV Forward Primer @ 7.5pmol/µL	1.0
PMTV Reverse Primer @ 7.5pmol/µL	1.0
PMTV Probe @ 5.0pmol/µL	0.5
Moloney Murine Leukaemia Virus(M-MLV) Reverse Transcriptase	0.05
Sterile H_2O	4.95
Sample RNA	1

*Sigma, Dorset, UK

Amplification and detection was performed on an Applied Biosystems 7900HT PCR System (Applied Biosystems, USA). The thermal cycle protocol for real-time RT-PCR

amplification was as follows: 48°C for 30 minutes, 94°C for 2 minutes and 40 cycles of 95°C for 15 seconds plus 60°C for 1 minute. Each sample was analysed in triplicate. Positive and negative controls were included in each assay. To monitor non-specific amplification, no template controls (NTC) with 1µL water instead of RNA template were also included in each analysis.

4.2.2.4.3 Preparation of positive and negative control material from tuber tissue

Total RNA was extracted from tuber tissue that had been previously tested for PMTV by ELISA. Tubers that had tested negative for PMTV infection by ELISA were used as negative control material. Tissue showing symptoms of PMTV infection (spraing) was excised, placed in a Bioreba bag (Bioreba AG, Switzerland) and ground in 2mL grinding buffer (Section 2.2.3) using a Homex 5 homogeniser (Bioreba AG, Switzerland). Five hundred microlitres of the homogenised material was added to a microtube with 500µL of 6M potassium acetate. The tube was vortexed and incubated on ice for 15 minutes prior to centrifugation at 11,000g for 15 minutes. Approximately 700µL supernatant was transferred to a fresh microtube containing 700µL 4M LiCl. The tube was vortexed and incubated at 4°C overnight. The tube was then centrifuged at 11,000g for 25 minutes to pellet the RNA. The supernatant was then discarded and the RNA pellet was resuspended in 1mL lysis buffer (Toyobo, Japan) prior to total RNA extraction using the Kingfisher™ system (Thermo Labsystems, Finland) and Magextractor®-RNA-reagents (Toyobo, Japan) (see section 4.2.2.3). Extracted RNA template was stored at -20°C prior to testing by real-time RT-PCR.

4.2.2.5 Testing field soils using the bait assay

The aim of this part of the study was to determine the importance of soil inoculum in causing economic outbreaks of PMTV infection in a crop, to establish whether there is a difference in inoculum levels in a field prior to planting and after harvesting the crop and how these relate to tuber infection in the crop. Another objective was to determine whether there are areas of a field which are 'hotspots' or if the inoculum is homogeneously distributed across a field. All sample soils were tested for PMTV using the method described in Section 4.2.2.

4.2.2.5.1 Sampling field soil

When sampling fields over 4 hectares in size, the area was split into units of 4 hectares or less for sampling purposes. The units to be sampled were walked in an extended 'W' pattern (Figure 4-3) and sub samples (cores) were taken using a 5 cm x 1 cm borer at various points covering the entire unit of the 'W' shaped walk. The number of cores taken from each sample unit varied between sites with wet, sticky, clay soils requiring fewer cores per sample than dry, sandy, clay soil which did not core as well. In total, *c.* 600mL of soil was collected from each sample unit. Each soil sample was then dried as described in Section 4.2.2.2.

4.2.2.5.2 Cara survey soil samples

Soil samples were collected from all land being used to grow crops of cv. Cara largely for use as certified farm saved seed potatoes (Table 4-2). Soil was sampled from crops in mid-August, 2004. Cores were taken from the drills of the crop when the plants were nearing senescence. In 2006, soil samples were taken from all 28 fields (Table 4-4) prior to planting and again after harvest the following spring.

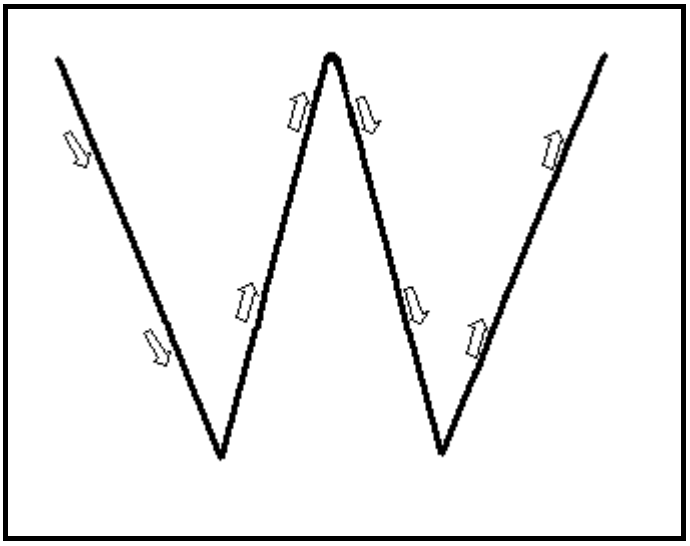

Figure 4-3. 'W' shaped walk typically used for sampling field soils. Approximately 100 cores are taken using a 5 cm x 1 cm borer at various points covering the entire unit of the 'W' shaped walk and bulked to give one sample.

4.2.2.5.3 Determining the distribution of PMTV inoculum in fields

Two crops of cv. Cara from Central Scotland in 2004 (Table 4-2) were found to have a considerably higher incidence of PMTV infection than other crops (Table 4-9). These crops were found to have 52% and 35% PMTV infection respectively. In order to assess the distribution of inoculum in the fields used to grow the crops, soil samples were collected from both sites on 10 March, 2006 and tested for PMTV. Only areas where crops of cv. Cara were grown in 2004 were sampled. Geographical co-ordinates (British National Grid) of the perimeter of the site were recorded using a hand held GPSMap 76 versatile navigator receiver (Garmin Europe Ltd, UK). The area was divided into quadrants and GPS co-ordinates were also recorded for the perimeter of each quadrant. Each quadrant was treated as a separate site for sampling purposes (see 4.2.2.5.1). GPS co-ordinates were entered into Arcview GIS 8.3 software and maps of the field layout were produced.

4.2.2.6 Temperature and PMTV from soil inoculum

This experiment was conducted to determine the importance of temperature on virus infection, symptoms in the tuber flesh and the development of powdery scab lesions in tubers of 5 cultivars. This experiment was conducted using adjacent glasshouse units at SASA. The temperature in each glasshouse was controlled and monitored electronically (Ecotech (UK) Ltd). This software system controls the temperature to within ± 2^{o}C of the set temperature by managing roof and side blinds, fans drawing in outside air and heaters within each unit. An alarm is activated in the event that the temperature limits are exceeded. There were no such events in the course of this experiment.

4.2.2.6.1 Preparation of seed tubers

Tubers of cultivars established as being susceptible to infection by PMTV in seed transmission experiments (cvs Nicola, Cara, Slaney, Rooster and Saturna) were selected from seed potatoes produced at SASA's Gogarbank Farm in 2006 and tested for PMTV by ELISA as described in section 2.2.4. All tubers tested negative for PMTV. Prior to planting in pots, the tubers were covered with paper towel and stored at room temperature for 3 weeks to chit. Once chitted, the tubers were planted.

4.2.2.6.2 Soil Preparation

Soil was collected from a field near Auchterarder, Perthshire which was known to be infested by PMTV. The infested soil was mixed with John Innes Compost No. 2 (1:1) in a cement mixer to give a homogeneous sample. Pots were filled with either the infested soil/compost mixture or compost alone.

4.2.2.6.3. Experimental Design

PMTV-free tubers of five cultivars were planted in 7 litre pots in either the soil-compost mixture or unamended compost. Each tuber was planted to a depth of 7cm in the pots on 7 February, 2007 and watered generously on a daily basis to encourage infection by zoospores of *S. subterranea*. Soil moisture sensors (SM200) (Delta-T Devices Ltd, Cambs, UK) were placed in the soil and soil moisture was monitored daily using a GP1 Data logger (Delta-T Devices Ltd, UK) as a basis for determining the watering regime. Plants were grown under natural light conditions in 3 different glasshouses at a constant

temperature of either 12°C, 19°C or 26°C. In each glasshouse, pots were laid out on the middle bench in a randomised block layout with 5 replications. Haulm was manually removed by cutting stems at soil level on 8 May, 2007 because there was evidence of new growth from daughter tubers in the 19°C and 26°C glasshouses. Plants in all three glasshouses were harvested individually into new paper bags on 22 May, 2007. Daughter tubers were held in a refrigerated store (4°C (±2°C)) for 2 weeks prior to being subject to temperature treatments to induce spraing (section 2.2.2). All daughter tubers were tested for PMTV by ELISA (Section 2.2.3) and sliced for examination for spraing symptoms. Tubers were scored as slightly, moderately or severely affected by spraing according to ADAS key 2.6.1 for potato spraing caused by TRV (Figure 4-4) (Anon, 1976) (section 4.2.1.3) and visually assessed for surface area coverage by powdery scab lesions (section 4.2.1.2), using a scale devised by Merz (2000) (Figure 4-5). Results are given as the mean severity score of affected tubers only for each sample.

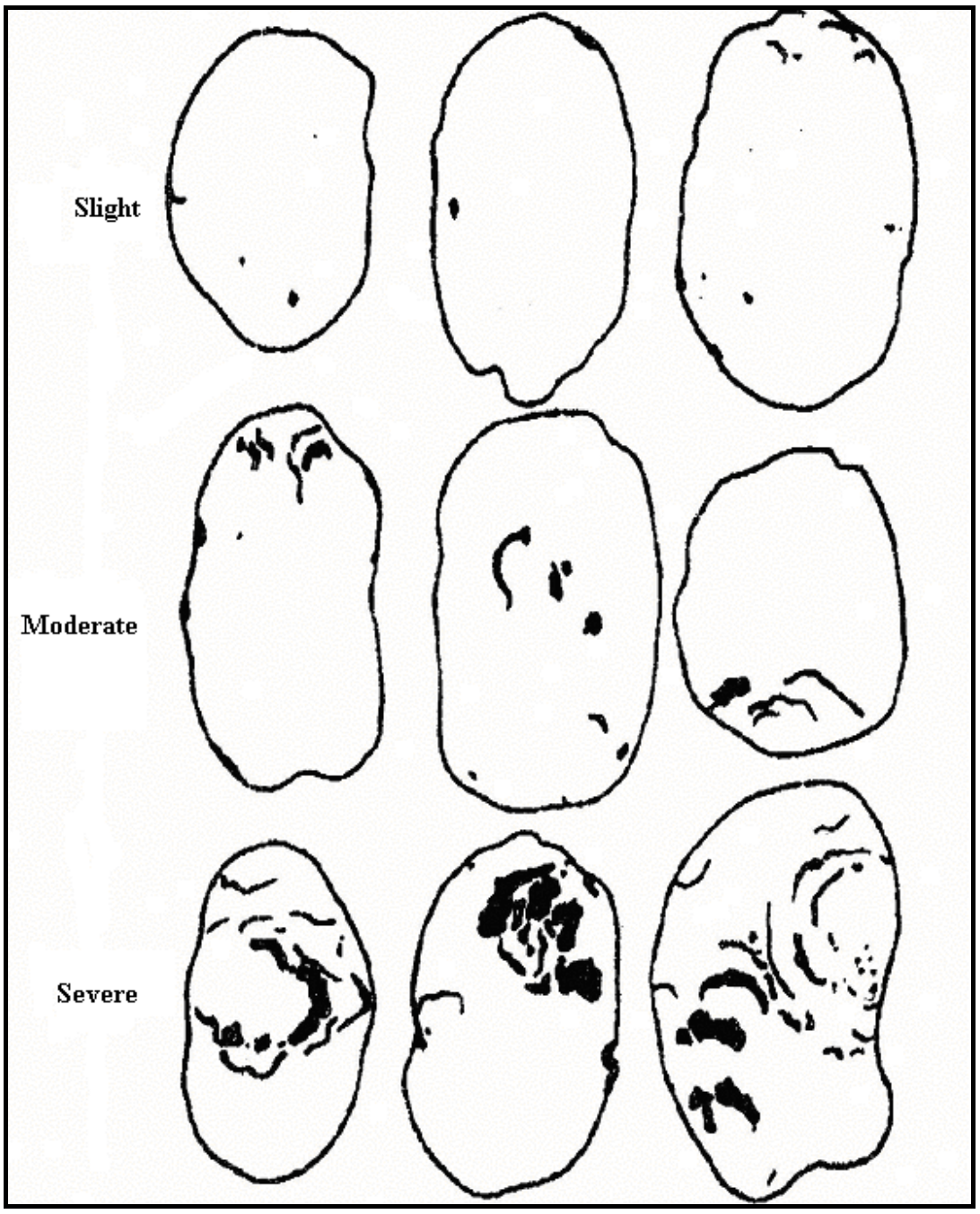

Figure 4-4. Key 2·6·1 used to determine the severity of spraing symptoms in the tuber samples (Anon, 1976).

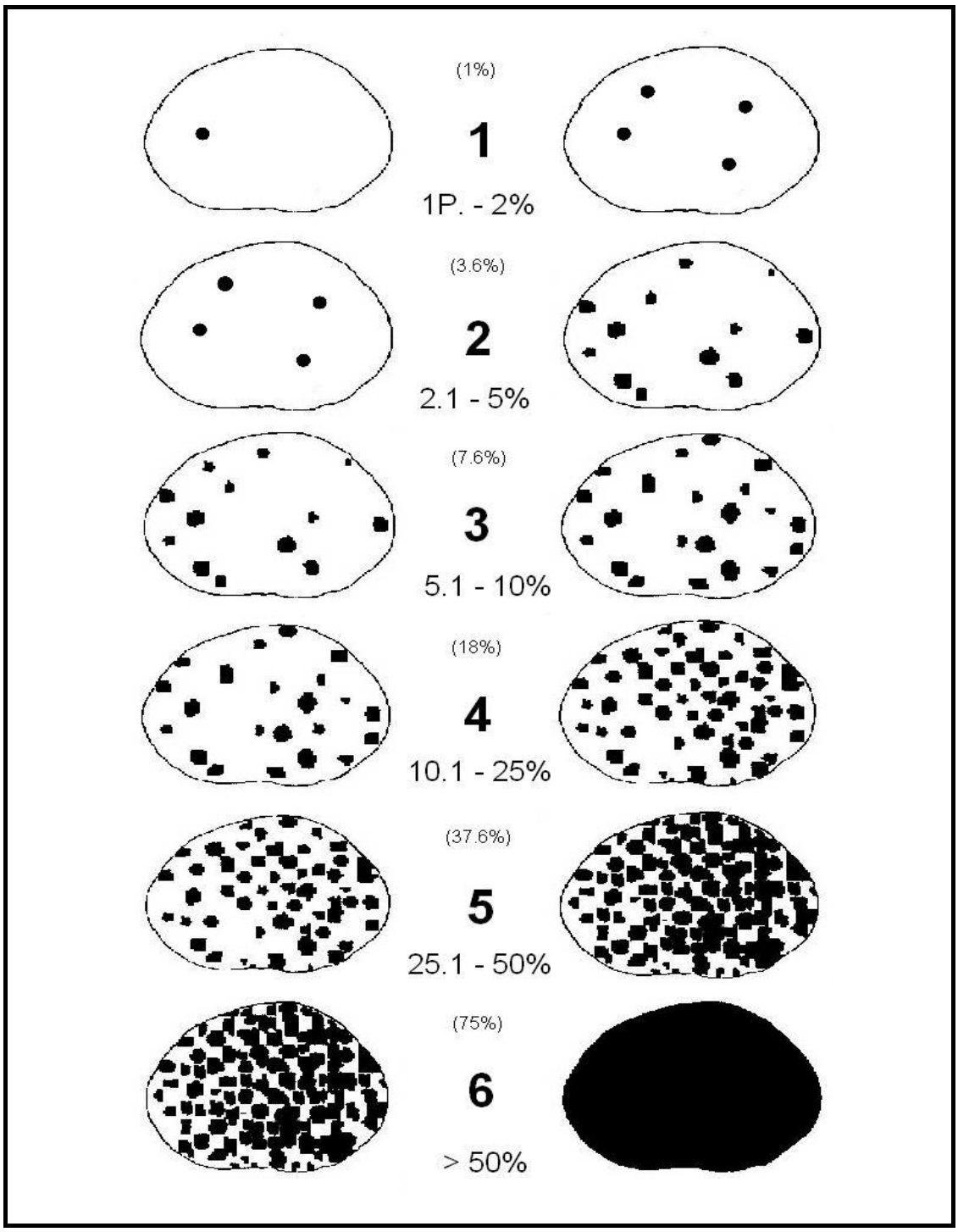

Figure 4-5. Standard powdery scab scoring table used to assess the severity of the lesions on the tubers (Merz, 2000).

4.2.3 Statistical analysis

Pearson's *r* correlation coefficient was calculated to study the linear correlation between soil inoculum and PMTV infection, symptom expression and infection and the occurrence of powdery scab and PMTV infection. Values of Pearson's *r* close to 0 indicate little or no correlation; whereas, values approaching 1 denote a positive correlation. Correlation analysis was conducted using Microsoft Excel 2003.

The efficiency of both RNA 2 and RNA 3 primer sets were compared using the Kappa statistic (K). The Kappa statistic (Cohen, 1960) compares the agreement between both primer sets against that which may be expected by chance. Possible values range from +1 (perfect agreement) via 0 (no agreement) to -1 (complete disagreement).

Logistic regression analysis, as discussed in Section 2.2.5, was conducted on variables recorded in the glasshouse trials. On occasions, a comparison between treatments was made using Student's *t* – test for paired samples of means.

4.3 Results

4.3.1 Study of seed and soil transmission

In order to determine the relative importance of seed and soil-borne inoculum in causing PMTV infection in potato crops in Scotland, the incidences of PMTV, spraing and powdery scab in seed potato crops of cv. Cara derived from a common seed source and grown at a wide range of sites in Eastern Scotland, were monitored over a 3 year period. The initial seed potatoes were multiplied to class SE 1 or 2 by a single grower in the North of Scotland before being distributed to a range of other growers, mainly in Angus, Perthshire and Aberdeenshire. These growers multiplied the seed potatoes for a further 2 years. In the first year, the growers mainly plant potatoes to produce further seed potatoes for re-planting in year 2. Year 2 seed potatoes are then marketed as class E1 seed potatoes to UK and other markets. The health of seed and daughter tubers from these crops was studied over two multiplication seasons; 2004-2005 and 2005-2006. For the purpose of presenting the results, each grower was assigned a unique number. Farm numbers only indicate different farms in any year.

4.3.1.1 2004 study

Seed potatoes from one crop of class SE1 and one crop of class SE2 were distributed by the initial grower to 31 farms for multiplication in 2004. In both crops of seed potatoes, the incidence of PMTV and powdery scab was 0.5% and 5.8% respectively. Of the 31 daughter crops produced from the seed tubers (Table 4-9, Figure 4-6), only one had no powdery scab on daughter tubers; however, PMTV was detected in the daughter tubers of this crop (Angus/27/1) but at the same incidence as in the planted seed potatoes. The highest incidence of powdery scab (85.6%) was found in a Perth-grown crop (Perth/9/1). A high incidence of PMTV (34.9%) was also detected in this crop, along with a correspondingly high incidence of spraing affected tubers (31.2%). However, another crop (Angus 25/1) had 55 % of tubers affected by powdery scab but no infection by PMTV. Most of the 31 daughter crops (90%) derived from the initial seed potatoes had <4% PMTV infection; however, the remaining 10% had much higher incidences of PMTV and equally high incidences of spraing affected tubers; the greatest being 52% for Perth/31/1 (both spraing and PMTV infection). There was a strong relationship between the incidence of PMTV and the incidence of spraing in the

daughter crops (r = 0.98, 29 d.f.) (Figure 4-6); however, no correlation was evident between the incidence of PMTV and the incidence of powdery scab (r = 0.40, 29 d.f.).

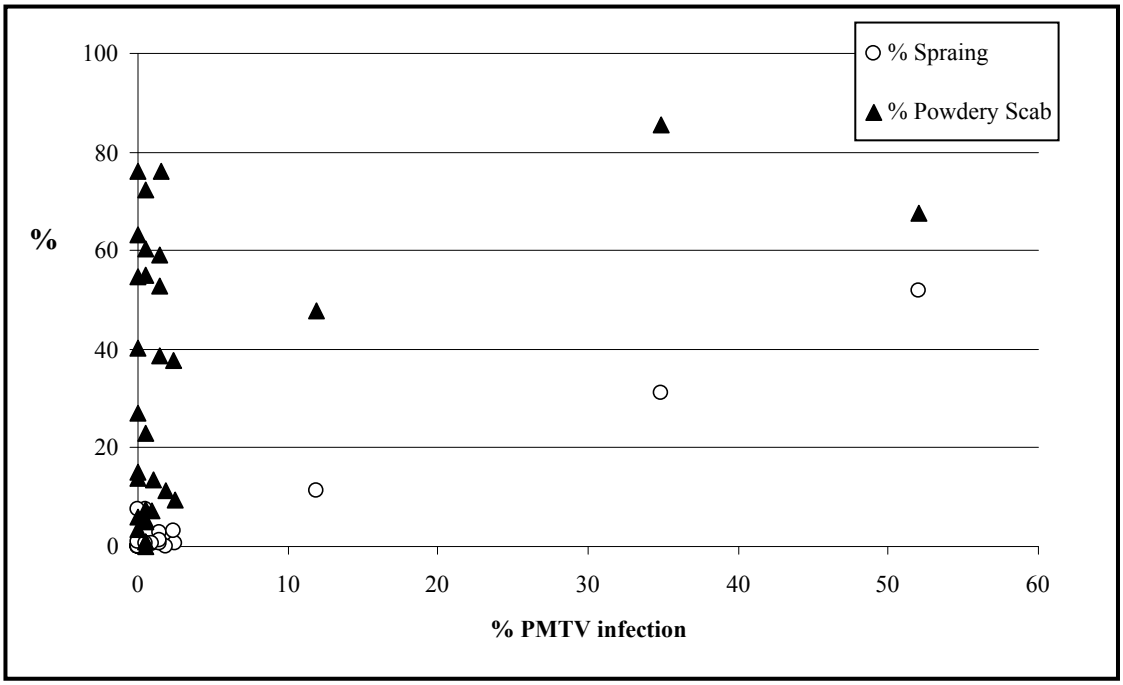

Figure 4-6. Incidence of powdery scab, PMTV and spraing in daughter tubers from 31 crops of cv. Cara produced in 2004 from common origin seed.

Table 4-9. Percentage PMTV, spraing and powdery scab in the 31 crops of cv. Cara tested in 2004.

*Area/Grower/Farm	Entry grade at classification	% PMTV	% Spraing	% Powdery Scab
Banff/28/1	SE3	0.5	0	5
Angus/25/1	SE3	0	0	54.8
Angus/27/1	SE3	0.5	0	0
Angus/17/1	SE3	0	0	27
Angus/2/1	SE3	1	0.5	13.6
Angus/8/1	SE3	0	0	15.2
Angus/13/1	SE3	0.5	0.5	60.5
Angus/33/1	SE3	1.4	2.9	38.8
Angus/34/1	SE3	0	0	13.7
Angus/24/1	SE3	0.5	0.5	23
Angus/11/1	SE3	0.5	0.5	5.5
Angus/22/1	SE3	0	0	6
Angus/14/1	SE3	0.5	0.5	7.6
Perth/6/1	SE3	0.5	7.6	72.4
Angus/20/1	SE3	0.5	0.5	55
Perth/10/1	SE3	0	0	76.1
Perth/19/1	SE3	0	0	3.5
Perth/9/1	SE3	34.9	31.2	85.6
Perth/3/1	SE3	0.5	0.5	1
Fife/23/1	SE3	0	7.6	63.3
Aberdeen/21/1	SE2	0	1	40.3
Angus/5/1	SE2	11.9	11.4	47.9
Angus/30/2	SE2	2.5	0.5	9.5
Angus/26/1	SE2	1.5	0.5	76
Angus/18/1	SE2	1.9	0	11.4
Angus/12/1	SE2	1.4	0.5	52.8
Angus/7/1	SE2	2.4	3.3	37.6
Perth15/1	SE2	1.4	1.4	59.1
Perth/31/1	SE2	52	52	67.5
Fife/16/1	SE2	0.9	0.5	7.1
Moray/4/1	SE2	0.5	0.5	1

* Area/grower identity is constant. Farm identity may change each year as many of the growers may rent land from other farms.

4.3.1.2 2005 study

Seed potatoes from three of the crops studied in 2004 (Perth/31/1, Angus/33/1 and Angus/34/1) were not planted in 2005 and these growers purchased SE3 seed potatoes from other 2004 seed growers (Angus/8/1, Angus/21/1 and Angus/26/1). Table 4-10 shows the incidence of PMTV, powdery scab and spraing in all the 2005 crops and the corresponding seed crops. Of the 84 crops of cv. Cara grown in 2005, 27(32%) were derived from seed potatoes provided by the initial grower (Cromarty/1/1-3), 54 (64%) were derived from farm-saved classified seed or SE purchased seed potatoes. The incidence of PMTV in seed potatoes from the Cromarty grower was 0.7%. Table 4-10 shows the incidence of PMTV, powdery scab and spraing in all the 2005 crops and the corresponding seed crops.

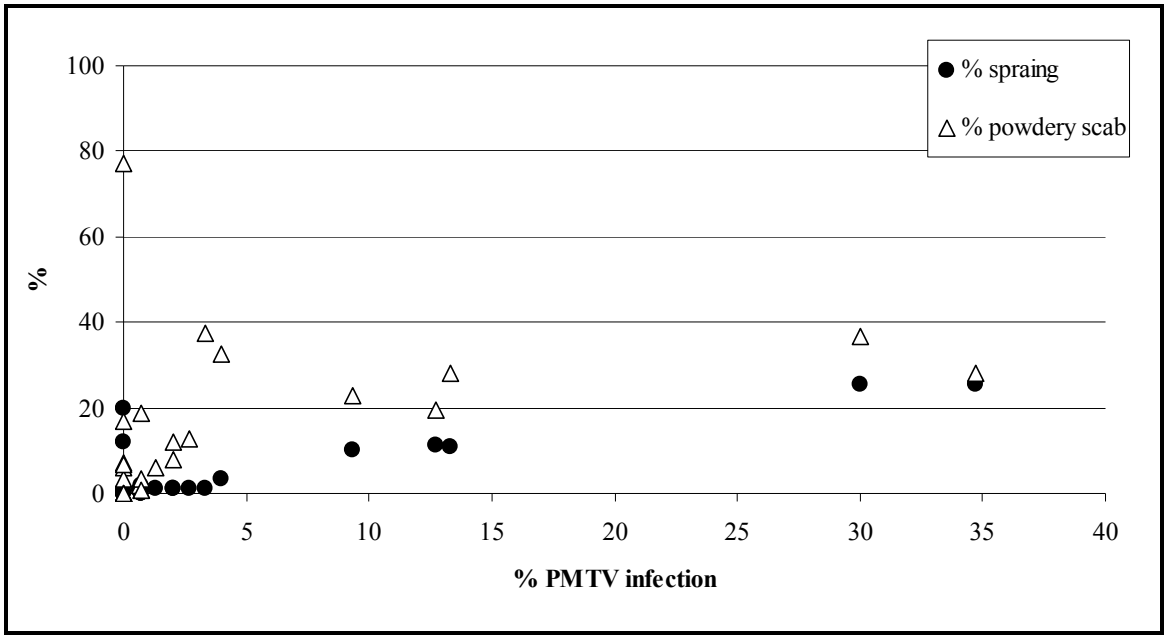

Figure 4-7. Incidence of powdery scab, PMTV and spraing in daughter tubers of 27 crops of cv. Cara grown in 2005 by the initial producer in Northern Scotland (Cromarty/1/1-3).

Of the 27 crops produced from the initial seed potatoes, relatively high amounts of PMTV occurred in two (30% (Angus/12/2) and 35% (Perth/10/1)) (Figure 4-7). The highest incidence of powdery scab (77%) in these 27 crops was observed on tubers from a Perth-grown crop (Perth/9/2); however, PMTV was not detected in these tubers and the incidence of spraing was low (0.7%).

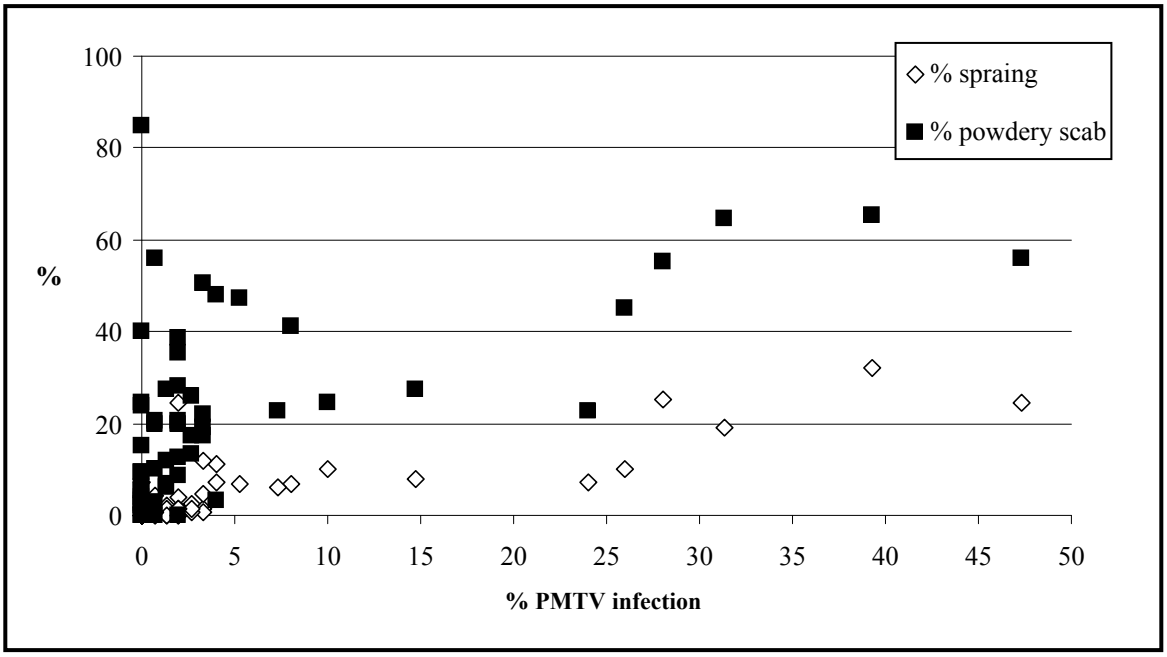

Figure 4-8. Incidence of powdery scab, PMTV and spraing in daughter tubers of the 57 crops of cv. Cara grown in 2005 from farm saved seed.

The incidence of PMTV in eight of the remaining 57 crops ranged from 10-47%. All eight of these daughter crops were derived from seed that contained less than 3% PMTV infection. Generally, the incidence of spraing in a crop was broadly similar to the amount of PMTV found in a crop ($r = 0.81$, 56 d.f). The highest incidence of powdery scab was 84.7% but no PMTV was detected in this crop (Angus/14/2c). As in 2004, the incidence of powdery scab in a crop was poorly correlated with the incidence of PMTV ($r = 0.57$, 56 d.f.).

Table 4-10. Percentage PMTV, spraing and powdery scab in the 84 crops of cv. Cara tested in 2005 and incidence of PMTV, spraing and powdery scab in the seed tubers.

2004				2005			
***Area/Grower/Farm**	**% PMTV**	**% spraing**	**% powdery scab**	***Area/Grower/Farm**	**% PMTV**	**% spraing**	**% powdery scab**
Cromarty/1/1	0.7	0	NA	Angus/6/2a†	0	20	16.7
				Aberdeen/29/1	0	12	0
				Aberdeen/29/2	0.7	0	18.7
				Angus/13/1a	2.7	1.3	12.7
				Angus/17/1	0	0	0
				Angus/18/2a	0.7	2	0.7
				Angus/30/1a	1.3	1.3	6
				Angus/12/2a	30	25.3	36.7
				Angus/11/2a	0.67	0.7	2
				Angus/7/2	0	0.7	6
				Angus/2/2a	3.3	1.3	37.3
				Angus/14/2a	2	1.3	12
				Fife/23/2	0	0	0
				Fife/16/1a	4	3.3	32.7
				Perth/10/1a	34.7	25.3	28
				Perth/31/1	2	1.3	8
Cromarty/1/2	0.7	0.7	NA	Angus/22/1a	0	0	3.3
				Angus/6/2b	13.3	10.7	28
				Moray/4/1a	0	0	0
				Perth/9/2a	0	0.7	77.3
				Perth/31/2	9.3	10	22.7
Cromarty/1/3	0.7	0	NA	Aberdeen/21/1a	0	0	7.3
				Angus/27/2a	0	0	0

* Area/grower identity is constant. Farm identity may change each year as fields can be rented.
† Letters denote crops grown at one farm, by one grower but in different fields.

Table 4-10 continued. Percentage PMTV, spraing and powdery scab in the 84 crops of cv. Cara tested in 2005 and incidence of PMTV, spraing and powdery scab in the seed tubers.

2004				2005			
*Area/Grower/Farm	% PMTV	% spraing	% powdery scab	*Area/Grower/Farm	% PMTV	% spraing	% powdery scab
Cromarty/1/3 contd.	0.7	0	NA	Angus/25/2	12.7	11.3	19.3
				Banff/28/1a†	0.7	0	3.3
				Perth/3/2	0.7	0	0.7
				Perth/19/2a	0	0	6.7
Angus/2/1	1	0.5	13.6	Angus/2/2b	1.3	2	27.3
				Angus/2/3a	8	6.7	41.3
				Angus/2/3b	3.3	1.3	22
Perth/3/1	0.5	0.5	0.1	Perth/3/3	0	0	9.3
				Perth/3/4	0	0	15.3
				Perth/3/1a	0	0	9.3
				Perth/3/1b	0	0	2
Moray/4/1	0.5	0.5	0.1	Moray/4/2	10	10	24.7
				Moray/4/1b	0	0	4
				Moray/4/1c	0	0	2.7
Angus/5/1	11.9	11.4	47.9	Angus/7/1	3.3	4.7	19.3
Perth/6/1	0.2	7.6	72.4	Perth/6/1	4	11.3	48
				Perth/6/2	3.3	12	17.3
Angus/7/1	2.4	3.3	37.6	Angus/5/2	24	7.3	22.7
				Angus/5/3	2	1.3	20
Angus/8/1	0	0	15.2	Angus/7/3	5.3	6.7	47.3

* Area/grower identity is constant. Farm identity may change each year as fields can be rented.
† Letters denote crops grown at one farm, by one grower but in different fields.

Table 4-10 continued. Percentage PMTV, spraing and powdery scab in the 84 crops of cv. Cara tested in 2005 and incidence of PMTV, spraing and powdery scab in the seed tubers.

2004				2005			
*Area/Grower/Farm	% PMTV	% spraing	% powdery scab	*Area/Grower/Farm	% PMTV	% spraing	% powdery scab
Perth/9/1	34.9	31.2	85.6	Perth/9/2b†	2	0	38.7
				Perth/9/3	1.3	0	12
				Perth/9/4	0	0	0
Perth/10/1	0	0	76.1	Perth/10/1b	39.3	32	65.3
Angus/11/1	0.5	0.5	5.5	Angus/11/2b	0.7	0	20.7
				Angus/11/3	2	1.3	8.7
Angus/12/1	1.4	0.5	52.8	Angus/12/3a	1.3	1.3	6
				Angus/12/3b	3.3	0.7	50.7
				Angus/12/3c	47.3	24.7	56
				Angus/12/4a	2.7	2.7	13.3
				Angus/12/4b	2	4	12.7
				Angus/12/4c	7.3	6	22.7
				Angus/12/4d	0.7	4.3	2.8
				Angus/12/2b	28	25.3	55.3
				Angus/12/5a	0	0.7	0
				Angus/12/5b	0	0	6
Angus/13/1	0.5	0.5	60.5	Angus/13/1b	4	7.3	3.3
Angus/14/1	0.5	0.5	7.6	Angus/14/1a	0.7	0	56
				Angus/14/1b	31.3	19.3	64.7
				Angus/14/2b	2	24.7	0
				Angus/14/2c	0	0	84.7
Perth/15/1	1.4	1.4	59.1	Perth/15/1	2.7	0.7	17.3

* Area/grower identity is constant. Farm identity may change each year as fields can be rented.
† Letters denote crops grown at one farm, by one grower but in different fields.

Table 4-10 continued. Percentage PMTV, spraing and powdery scab in the 84 crops of cv. Cara tested in 2005 and incidence of PMTV, spraing and powdery scab in the seed tubers.

2004				2005			
*Area/Grower/Farm	% PMTV	% spraing	% powdery scab	*Area/Grower/Farm	% PMTV	% spraing	% powdery scab
Fife/16/1	1	0.5	7.1	Fife/16/1b†	1.3	0	6.7
Angus/17/1	0	0	27	Angus/17/3	2	1.3	28
				Angus/17/2	26	10	45.3
Angus/18/1	1.9	0	11.4	Angus/18/2b	14.7	8	27.3
Angus/30/2	2.5	0.5	9.5	Angus/30/1b	0	0	24
				Angus/30/1c	0	7.3	0
Perth/19/1	0	0	3.5	Perth/19/2b	0	0	3.3
Angus/20/1	0.5	0.5	55	Perth/20/2	0	0	24.7
				Angus/20/3	0.7	0.7	0
Aberdeen/21/1	0.5	0	5	Aberdeen/21/1b	0.7	0.7	20
Angus/22/1	0	0	6	Angus/22/1b	0	0.7	9.3
				Angus/22/2	0.7	0.7	10
Fife/23/1	0	7.6	63.3	Fife/23/3	0	0	2.7
				Fife/23/4	0	2.7	5.3
Angus/24/1	0.5	0.5	23	Perth/9/5	2	0	20.7
Angus/25/1	0	0	54.8	Angus/25/3	2.7	1.3	26
Angus/26/1	1.5	0.5	76	Roxburgh/32/1	2	1.3	35.3
Angus/27/1	0.5	0	0	Angus/27/2b	0	0	5.3
Banff/28/1	0.5	0	5	Banff/28/1b	0	1.3	40

* Area/grower identity is constant. Farm identity may change each year as fields can be rented.
† Letters denote crops grown at one farm, by one grower but in different fields.

The incidence of PMTV infection in the seed crops did not correlate with the incidence of spraing, powdery scab or PMTV infection in the daughter crops (r = 0.02, 30 d.f., r = 0.005, 30 d.f., r = 0.009, 30 d.f. respectively) (as shown in Figures 4-7 and 4-8). This can be further demonstrated by examining the occurrence of PMTV tuber infection in crops over 3 generations of multiplication, for example, the seed crop (Perth/9/1), with the highest incidence of PMTV infected tubers (35%) in 2004, produced three daughter crops which had a low incidence of PMTV infected tubers (2%, 1% and 0% respectively) (Figure 4-9), were all free from spraing symptoms, and all had a low incidence of powdery scab. Another example is shown Figure 4-10 in which a high incidence of PMTV infection in tubers in 1 out of 4 crops derived from seed potatoes containing 0.5% PMTV tuber infection. As with the 2004 crops, there was no relationship between the incidence of PMTV and the occurrence of powdery scab on daughter tubers (r = 0.27, 83 d.f.). There was a relationship between the incidence of PMTV and the incidence of spraing in the daughter tubers (r = 0.65, 83 d.f.); however, the relationship was not as strong as in 2004 (Figures 4-6 and 4-7).

The occurrence of spraing symptoms in the seed crops was not correlated with the incidence of spraing in the daughter tubers (r = 0.008, 30 d.f.), nor was the incidence of PMTV infection in the daughter crops correlated with the incidence of spraing in the seed crops (r = 0.01, 30 d. f.). For example, 20% of the daughter crops derived from a crop grown in Central Scotland which had 0.5% spraing (Angus/12/1) were free from spraing symptoms. A further 20% of the daughter crops had approximately 25% tubers affected by spraing and the remaining 60% had less than 10% tubers with spraing symptoms. The progeny (Angus/17/3 and Angus/17/2) of seed potatoes of another 2004 Angus grown crop (Angus/17/1) were also affected by spraing symptoms in the tubers (1% and 10% respectively) even though spraing symptoms were absent in the seed crop.

Powdery scab symptoms were not observed on 12% of the 84 crops studied in 2005 (Figure 4-7). There was no correlation (r = 0.04, 27 d.f.) between the incidence of powdery scab on seed tubers and the incidence of powdery scab on daughter tubers. The highest incidence of powdery scab was observed on daughter tubers of a crop grown in Central Scotland (Angus/14/2c), with 85% of tubers being affected; although the incidence of powdery scab

on seed tubers was relatively low (7.6%). The three daughter crops derived from seed potatoes of one 2004 crop (Perth/9/1) which had 86% of tubers affected by powdery scab, were not affected by powdery scab to the same extent as the seed tubers. One daughter crop (Perth/9/4) was unaffected by powdery scab, whereas the other two (Perth/9/2b and Perth/9/3) had 39% and 12% of daughter tubers affected, respectively.

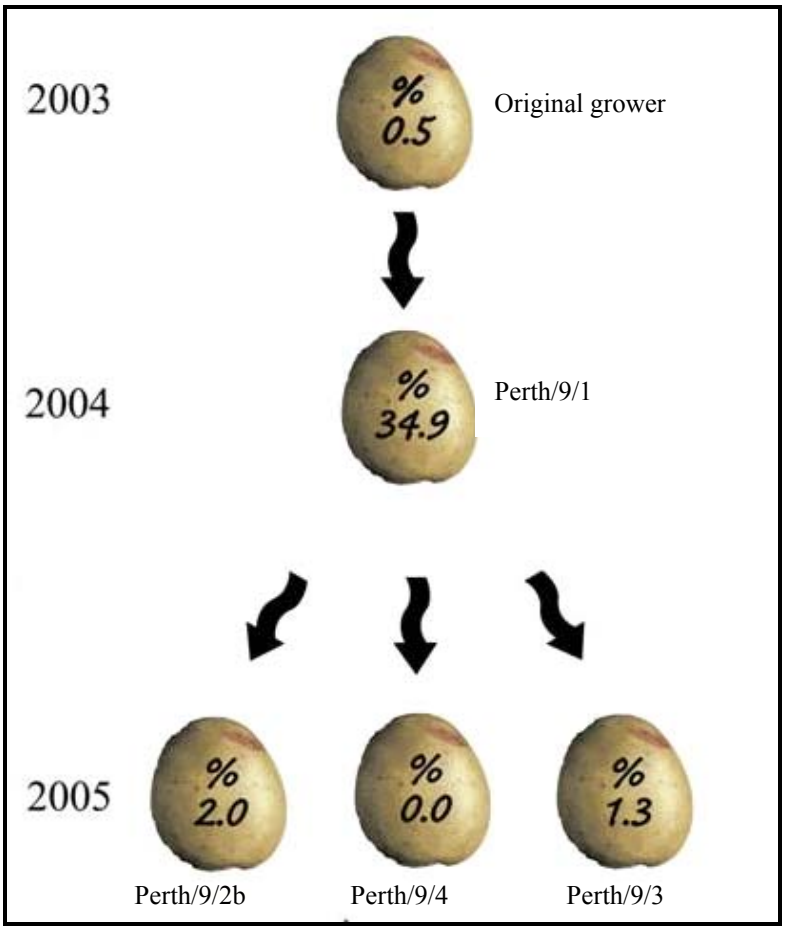

Figure 4-9. Pattern of PMTV tuber infection in potatoes over 3 field generations of seed multiplication for a number of farms in Perthshire. Crops produced in 2004 and 2005 were grown by the same grower albeit on different farms.

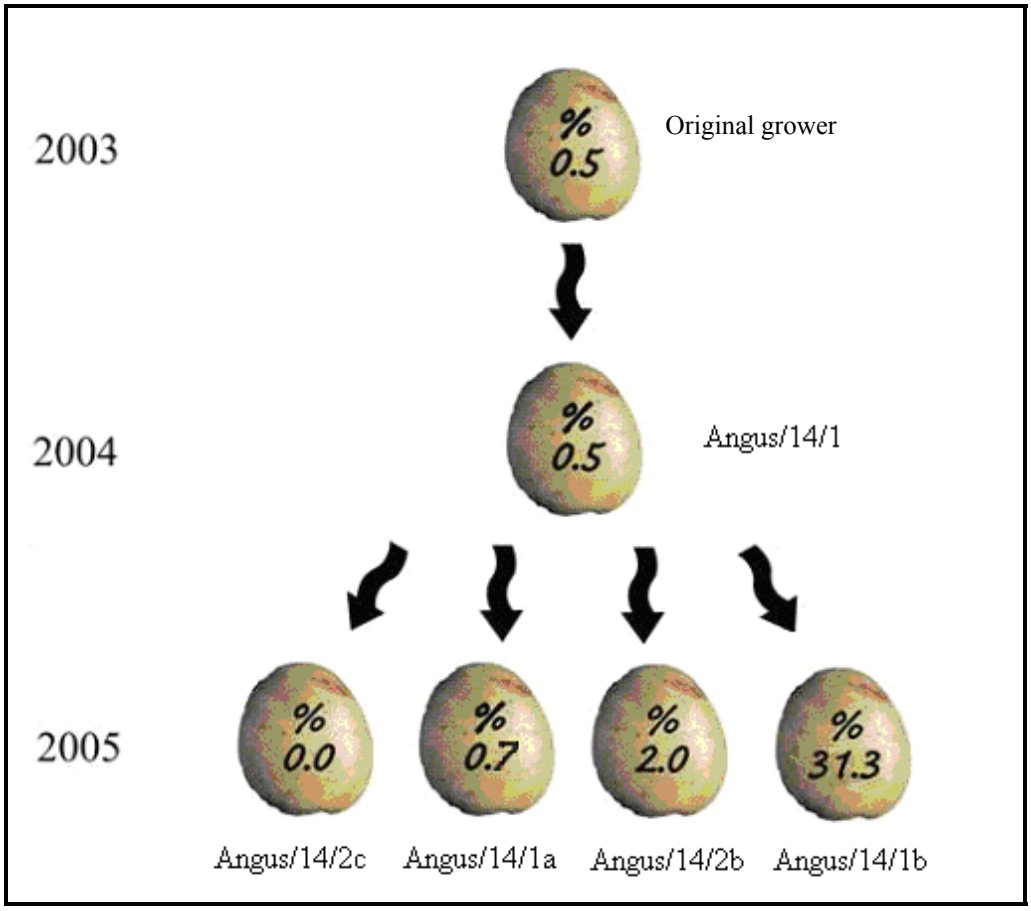

Figure 4-10. Pattern of PMTV tuber infection in potatoes over 3 field generations of seed multiplication for a number of farms in Angus. Crops produced in 2004 and 2005 were grown by the same grower albeit on different farms.

4.3.1.3 2006 study

All 28 crops of cv. Cara studied in 2006 were derived from seed potatoes from 18 farm-saved classified seed crops (Table 4-11). Results from PMTV testing and disease assessments are shown in Table 4-11 and illustrated in Figure 4-11. As with the 2004 and 2005 studies, there was no correlation between PMTV infection in the seed crops and PMTV infection in the daughter crops ($r = 0.008$, 17 d.f.) (Figure 4-12). Crop Perth/19/3 had a considerably higher incidence of PMTV (45%) than its sister crop (5%) although both were derived from the same PMTV-free seed. Crop Angus/25/2 (1.3% PMTV) was derived from a seed crop with almost 13% PMTV infection (Figure 4-10), whereas, Perth/31/2 produced a daughter crop with a comparable incidence of PMTV infection (9.3% and 8.7% respectively) (Table 4-11). High incidences of powdery scab did occur in

a few crops but were not linked to the incidence of powdery scab symptoms on the seed tubers (r = 0.01, 17 d.f.).

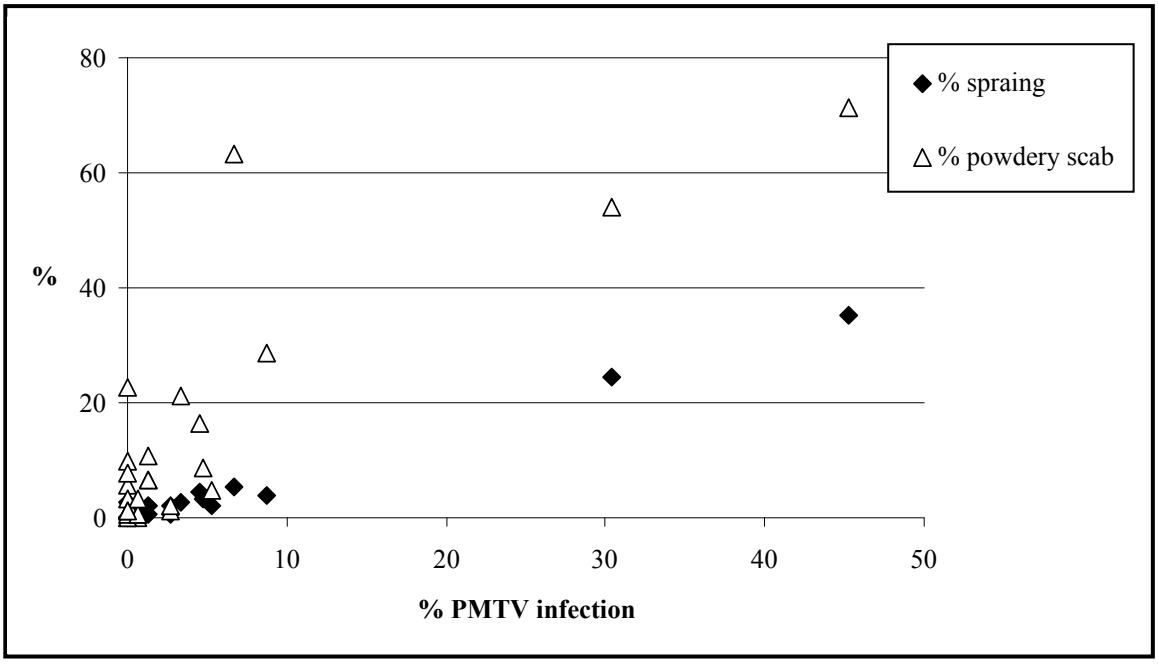

Figure 4-11. Incidence of powdery scab, PMTV and spraing in daughter tubers of 28 crops of cv. Cara grown in 2006.

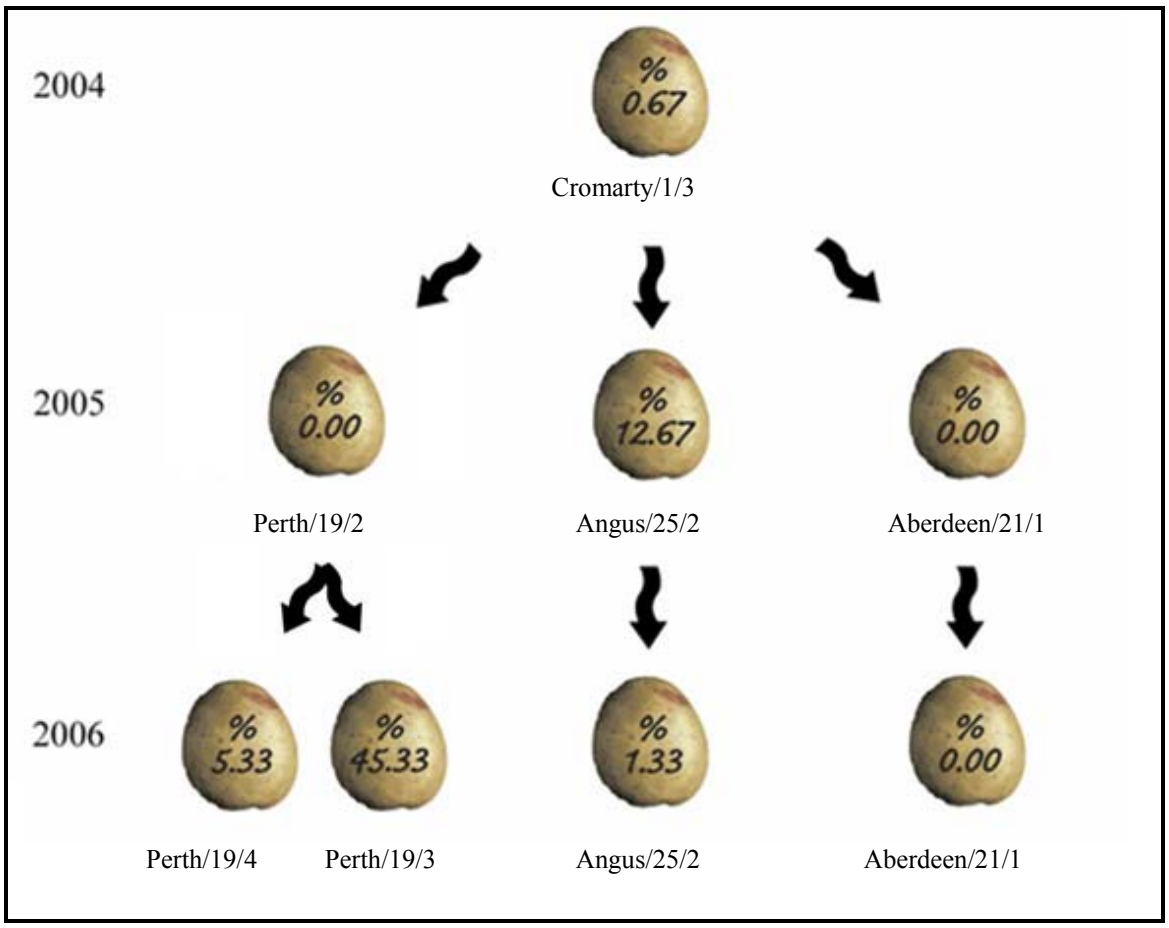

Figure 4-12. Pattern of PMTV tuber infection in potatoes over 3 field generations of seed multiplication for a number of farms in central and north-east Scotland.

The highest incidence of powdery scab on a 2006 crop was 71% (Perth/19/3); however only 7% of the seed tubers from the 2005 crop were affected by powdery scab (Perth/19/2). Perth/19/3 also had 45% of tubers infected with PMTV and 35% with spraing, in contrast to the 2005 seed crop where both were found to be absent. As with the 2004 and 2005 studies, the incidence of spraing in tubers was strongly correlated with PMTV infection ($r = 0.98$, 27 d.f.) but was not correlated with the incidence of PMTV or spraing in seed tubers ($r = 0.01$, 17 d.f. and $r = 0.02$, 17 d.f. respectively).

Table 4-11. Occurrence of PMTV, spraing and powdery scab in daughter tubers of 28 crops of cv. Cara grown in 2006 in relation to the incidence of seed infection in 2005, region and grower.

2005				2006			
*Area/Grower/Farm	% PMTV	% spraing	% powdery scab	Area/Grower/Farm	% PMTV	% spraing	% powdery scab
Perth/3/2	0.7	0.0	0.7	Perth/3/1	0.3	0.3	0.7
				Perth/3/2	0.0	0.0	5.7
Angus/6/2	13.3	10.7	28.0	Angus/6/2	4.7	3.3	8.7
				Angus/6/3	0.7	1.3	0.0
Angus/7/2	0.0	0.7	6.0	Angus/7/2	30.4	24.5	53.9
Perth/9/2	0.0	0.7	77.3	Perth/9/6	1.3	2.0	6.7
				Perth/9/5	1.3	2.0	6.7
				Perth/9/1	2.7	0.7	1.3
Angus/11/2	0.7	0.7	2.0	Angus/11/4	0.0	0.0	22.7
Angus/13/1	2.7	1.3	12.7	Angus/13/1	0.0	0.0	3.3
Angus/14/2	2.0	1.3	12.0	Angus/14/3	4.5	4.5	16.5
				Angus/14/4	2.7	2.0	2.0
Angus/17/1	0.0	0.0	0.0	Angus/17/1	0.0	0.0	0.0
				Angus/17/4	0.0	0.0	0.0
Perth/19/2	0.0	0.0	6.7	Perth/19/3	45.3	35.3	71.3
				Perth/19/4	5.3	2.0	4.7
Aberdeen/21/1	0.0	0.0	7.3	Aberdeen/21/1	0.0	0.0	0.7
Angus/22/1	0.0	0.0	3.3	Angus/22/2	0.0	0.0	1.3
				Angus/22/1	0.0	0.0	10.0
				Angus/22/3	0.7	0.0	0.7

* Area/grower identity is constant. Farm identity may change each year as fields can be rented.

Table 4-11 continued. Occurrence of PMTV, spraing and powdery scab in daughter tubers of 28 crops of cv. Cara grown in 2006 in relation to the incidence of seed infection in 2005, region and grower.

2005				2006			
*Area/Grower/Farm	% PMTV	% spraing	% powdery scab	*Area/Grower/Farm	% PMTV	% spraing	% powdery scab
Fife/23/2	0.0	0.0	0.0	Fife/23/5	0.0	2.7	7.7
				Fife/23/1	0.7	0.0	0.7
Angus/27/2	0.0	0.0	5.3	Angus/27/3	0.7	0.0	3.3
Banff/28/1	0.0	1.3	40.0	Banff/28/1	6.7	5.3	63.3
Angus/25/2	12.7	11.3	19.3	Angus/25/2	1.3	0.7	10.7
Aberdeen/29/2	0.7	0.0	18.7	Aberdeen/29/2	3.3	2.7	21.3
Perth/31/2	9.3	10.0	22.7	Perth/31/3	8.7	4.0	28.7
Angus/30/1	1.3	1.3	6.0	Angus/30/2	0.0	0.0	1.3

* Area/grower identity is constant. Farm identity may change each year as fields can be rented.

4.3.2 Cara survey soil samples

4.3.2.1 Results of soil bait assay optimisation experiments

As described in Section 4.2.2.2, a soil bait assay was developed to detect PMTV inoculum in soil. A range of host species were screened for their suitability in different growing media (Table 4-12). PMTV was readily detected in the roots of *Lycopersicum esculentum* cv. Moneymaker (tomato) grown for 2 weeks in infested soil or infested soil-compost mixtures. Detection of PMTV was also more consistent in this species than in *Nicotiana benthamiana* (Table 4-13) and seedlings were more amenable to transplanting.

Table 4-12. Mean Ct value obtained from real-time PCR assay for the detection of PMTV in the root tissue and leaves of four plant species grown in naturally infested field soil for 2 or 3 weeks.

	Mean Ct value†			
	2 weeks		3 weeks	
Host Plant	**Roots**	**Leaves**	**Roots**	**Leaves**
Lycopersicum esculentum cv. Moneymaker	21.8	32.8	18.8	ND
Nicotiana tabacum cv. White Burley	21.8	ND*	18.8	ND
Nicotiana debneyi	22.0	ND	20.5	ND
Nicotiana benthamiana	19.4	31.8	16.0	ND

* ND =PMTV RNA not detected
† real-time RT-PCR assay of Mumford *et al.* (2000) used throughout

Table 4-13. Detection of PMTV by real time PCR in 31 soil samples using tomato (*Lycopersicum esculentum* cv. Moneymaker) and *Nicotiana benthamiana* (N.b.) as bait plants.

No. of replicates out of 3 in which PMTV was detected	Tomato	N.b.
0	20	26
1	5	1
2	1	3
3	5	1

4.3.2.1.1 Assay sensitivity of the six primer/probe combinations

Positive control material was tested for the presence of all three RNA molecules using the six primer/probe combinations as described in Section 4.2.2.4. The amplification plot comparing all primer sets is shown in Figure 4-13. The established primer/probe combination is labelled RNA 3B (Mumford *et al*., 2000). The results from the amplification plot indicate that the RNA 3B primer/probe combination is more sensitive than the newly designed RNA 3A primers. The amplification plot also indicates that RNA 2 is readily detected in PMTV positive tissue, with the RNA 2B product present in greater quantities than the product generated using the RNA 2A primer set and the established RNA 3B primer set. It was therefore decided to compare the RNA 2B primer set with the RNA 3B primer set.

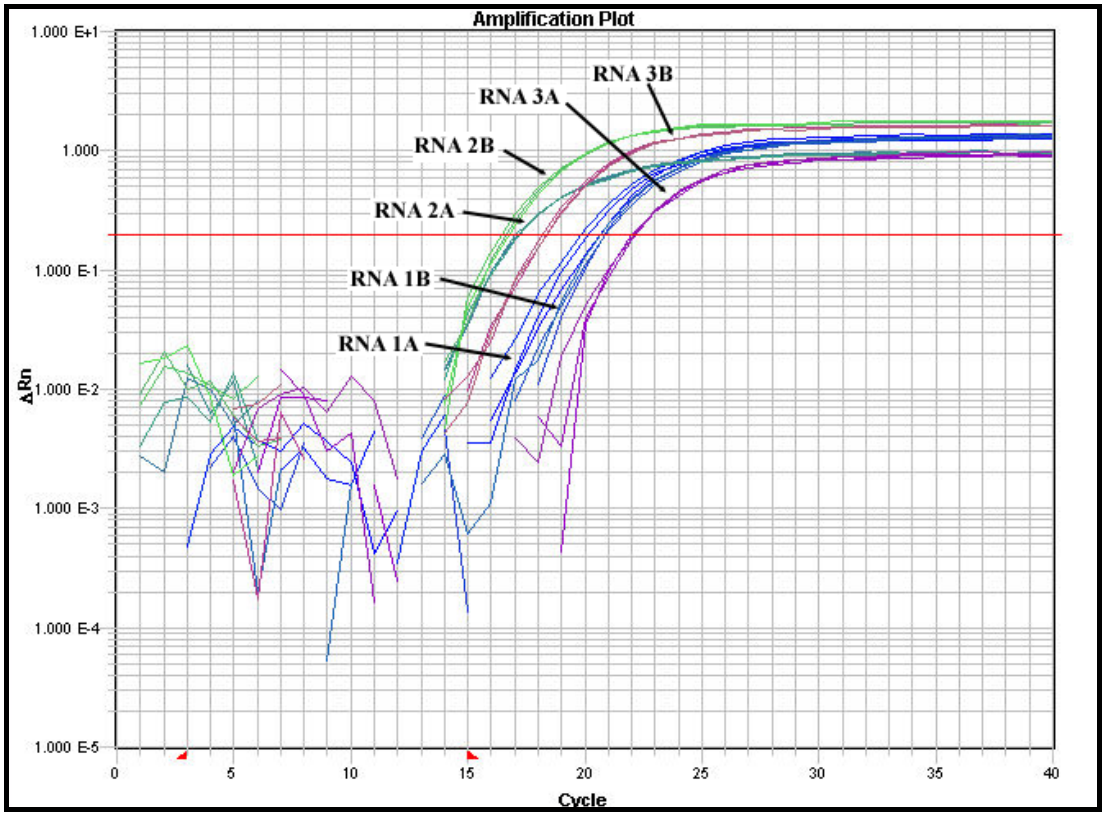

Figure 4-13. Real-time PCR amplification plot of PMTV detection made by comparing all six tested primer/probe combinations.

The sensitivity of the RNA 2B primers was compared with the previously used RNA 3B primer/probe combination. Standard curves were constructed using a serial dilution of infected tuber tissue. The standard curves are shown in Figures 4-14 and 4-15.

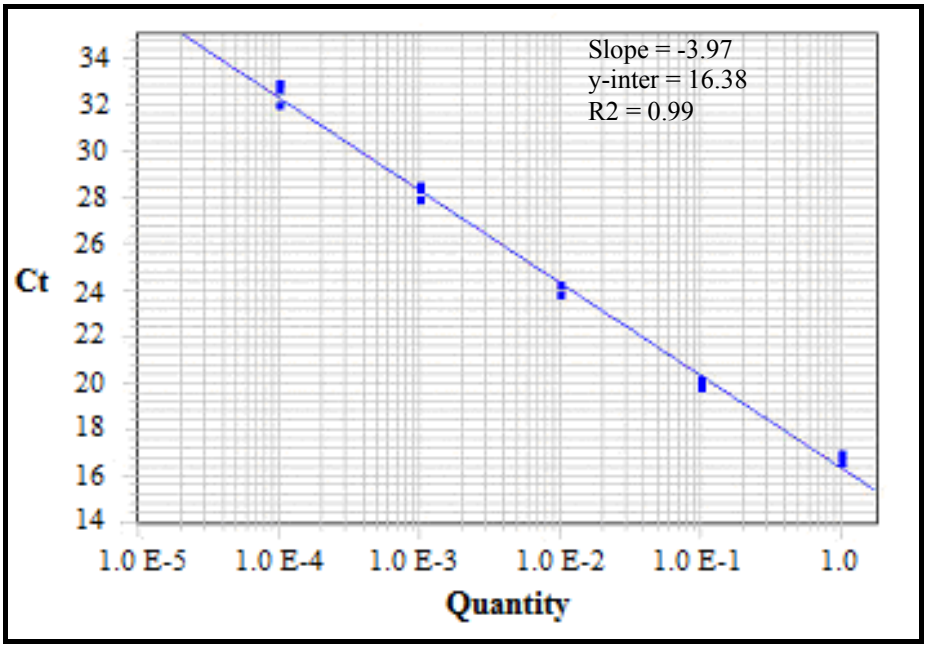

Figure 4-14. A serial dilution of PMTV RNA extracted from tuber tissue tested using the RNA 2B primer/probe combination.

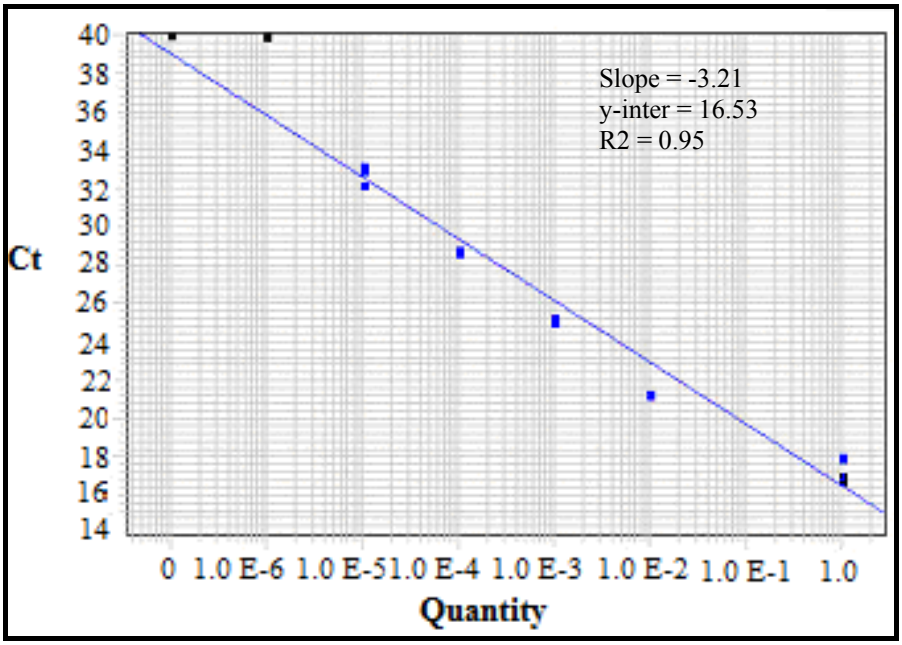

Figure 4-15. A serial dilution of PMTV RNA extracted from tuber tissue tested using the RNA 3B primer/probe combination designed by Mumford *et al*., 2000.

This standard dilution series was consistently detected using both primer sets; however the RNA 2B primer set consistently produced a linear correlation at least as good as the RNA 3B primer set.

4.3.2.2 2004 soil samples

Soil samples were collected from the 31 fields in which crops of cv. Cara were grown in 2004, as described in Section 4.2.2.5.1. The soil samples were collected from the drills of the crops in mid-August, when the crops were nearing senescence, and bulked to give one sample per field. Each soil sample was analysed for PMTV in triplicate (Table 4-14), as described in Section 4.2.2.2, to determine whether there was a correlation between the incidence of tuber infection by PMTV in a crop and the soil.

The percentage of PMTV tuber infection in the crops was not correlated with the occurrence of PMTV in the soil as expressed by the percentage of replicates in which PMTV was detected by either set of primers ($r = 0.32_{(30\ d.f)}$ for RNA 2, $r = 0.36_{(30\ d.f)}$ for RNA 3). PMTV was not detected by either set of primers in 20 of the 31 (65%) soils. For these 20 fields, infection by PMTV was detected in daughter tubers from 12 crops (60%). Of the 11 crops grown in soils in which PMTV was found, ten produced daughter tubers infected by PMTV, with infection ranging from 0.5% to 50%

The effectiveness of the two primer sets in detecting PMTV in soil samples was evaluated using replicate data for the 11 fields in which PMTV was detected (Table 4-15). The results for the two primer sets were in good agreement (K= 0.942), with differences found with only 2 replicates which tested positive using the RNA 2B primer/probe combination but were negative using the RNA 3B primer set.

Table 4-14. Occurrence of PMTV in 31 field soils in which seed crops of cv. Cara were grown in 2004 as assessed by a tomato bioassay and Real-time PCR using RNA 2B and RNA 3B primer/probe combinations.

		(RNA 3B Primers)			(RNA 2B Primers)		
Area/Grower/Farm	**% daughter tubers infected by PMTV**	**Rep 1**	**Rep 2**	**Rep 3**	**Rep 1**	**Rep 2**	**Rep 3**
Perth/31/1	52.00	+ (21.35)	+ (20.58)	+ (21.70)	+(16.27)	+(15.63)	+(17.13)
Perth/9/1	34.88	+ (18.66)	+ (18.82)	+ (17.83)	+(16.17)	+(16.20)	+(15.76)
Angus/5/1	11.85	+ (25.03)	+ (25.00)	+ (21.35)	+(18.08)	+(17.47)	+(15.50)
Angus/30/2	2.50	+ (23.69)	*-	-	+(20.57)	+(27.21)	-
Angus/7/1	2.38	-	-	-	-	-	-
Angus/18/1	1.90	-	-	-	-	-	-
Angus/26/1	1.50	-	-	-	-	-	-
Angus/33/1	1.44	-	-	+ (30.57)	-	-	+(26.09)
Perth15/1	1.43	+ (21.79)	+ (25.07)	+ (24.37)	+(17.60)	+(19.53)	+(20.19)
Angus/12/1	1.42	-	-	-	-	-	-
Angus/2/1	1.01	-	-	-	-	-	-
Fife/16/1	0.95	-	-	-	-	-	-
Angus/13/1	0.50	-	-	-	-	-	-
Angus/24/1	0.50	+ (27.79)	-	-	+(24.68)	-	-
Perth/3/1	0.50	-	-	-	-	-	-
Angus/11/1	0.50	-	+ (28.13)	-	+(28.68)	+(21.81)	-
Banff/28/1	0.50	-	-	-	-	-	-
Angus/14/1	0.48	+ (32.91)	-	-	+(28.36)	-	-
Angus/27/1	0.48	+ (18.60)	+ (18.07)	+ (20.35)	+(16.79)	+(16.19)	+(17.84)

* = PMTV RNA not detected. Figures in brackets denote the Ct value obtained from real-time PCR assay.

Table 4-14 continued. Occurrence of PMTV in 31 field soils in which seed crops of cv. Cara were grown in 2004 as assessed by a tomato bioassay and Real-time PCR using RNA 2B and RNA 3B primer/probe combinations.

		(RNA 3B Primers)			(RNA 2B Primers)		
Area/Grower/Farm	**% daughter tubers infected by PMTV**	**Rep 1**	**Rep 2**	**Rep 3**	**Rep 1**	**Rep 2**	**Rep 3**
Moray/4/1	0.48	*-	-	-	-	-	-
Angus/20/1	0.48	-	-	-	-	-	-
Perth/6/1	0.18	-	-	-	-	-	-
Fife/23/1	0.00	-	-	-	-	-	-
Perth/19/1	0.00	-	-	-	-	-	-
Angus/17/1	0.00	-	-	-	-	-	-
Perth/10/1	0.00	-	-	-	-	-	-
Angus/25/1	0.00	-	-	-	-	-	-
Angus/34/1	0.00	-	+ (25.72)	+ (23.50)	-	+(19.93)	+(18.75)
Angus/22/1	0.00	-	-	-	-	-	-
Angus/8/1	0.00	-	-	-	-	-	-
Aberdeen/21/1	0.00	-	-	-	-	-	-
+ve control soil	NA	+ (24.14)	+ (22.62)	+ (21.83)	+(17.44)	+(16.23)	+(15.41)
Compost	NA	-	-	-	-	-	-

* = PMTV RNA not detected. Figures in brackets denote the Ct value obtained from real-time PCR assay.

Table 4-15. Number of soil sample replicates in which PMTV was detected using RNA 2B or RNA 3B primers.

		RNA 3B		
		Positive	Negative	Total
RNA 2B	Positive	22	2	24
	Negative	0	69	69
	Total	22	71	93

K = 0.942

PMTV was detected in root tissue of bait plants in all 3 replicates of 5 of the field soil samples (Table 4-16); however, the incidence of tuber infection by PMTV in crops grown in these field soils differed considerably. Tuber infection in 3 of these crops (Perth/31/1, Perth/9/1 and Angus/5/1) was much greater than in the other 2 crops (Perth/15/1 and Angus/27/1) (Table 4-17). The replicate means for these five soils were used to examine whether the primer sets differed in the amount of RNA which they detected in the test samples. The mean Ct value of the RNA 2 primer/probe combination was lower than that obtained using the RNA 3 primer set ($t_{(4\ d.f.)} = 4.8$, $P < 0.01$) (Table 4-17).

Table 4-16. Number of PMTV positive soil samples (31 fields) in which cv. Cara was grown in 2004 as detected by bioassay and real-time PCR using RNA 2 or RNA 3 primers.

No. of replicates in which PMTV RNA was detected	RNA 2B	RNA 3B
0	20	20
1	3	5
2	3	1
3	5	5

Table 4-17. Mean Ct values for PMTV RNA obtained by real-time PCR for tomato bait plants grown in 5 field soils in which PMTV was detected in every replicate.

		Mean Ct value	
County/Grower/Farm	% PMTV infection	RNA 3B	RNA 2B
Perth/31/1	52.0	21.21	16.34
Perth/9/1	34.9	18.44	16.04
Angus/5/1	11.9	23.79	17.02
Perth15/1	1.4	23.74	19.11
Angus/27/1	0.5	19.01	16.94

4.3.2.2.1 Determining the distribution of PMTV inoculum in fields

As described in Section 4.2.2.5.3, a hand held GPS receiver was used to sub-divide two fields in central Scotland that produced seed potatoes of cv. Cara in 2004 with substantial amounts of infection by PMTV. The highest incidence of PMTV infection (52%) was found in Perth/31/1 crop and 35% of the sampled tubers from another Perth grown crop (Perth/9/1) were infected by PMTV. Soil was sampled from each quadrant as described in Section 4.2.2.5.1 and tested for PMTV using the tomato bait assay and real-time PCR assay described in Sections 4.2.2.2 and 4.2.2.3. Figures 4-16 and 4-17 illustrate the sections of the field in which PMTV was detected. PMTV was unevenly distributed within both sampling sites, being detected in 2 adjacent quadrants out of 4 in the Perth/31/1 sample site and in one quadrant out of 4 in the Perth/9/1 sample site.

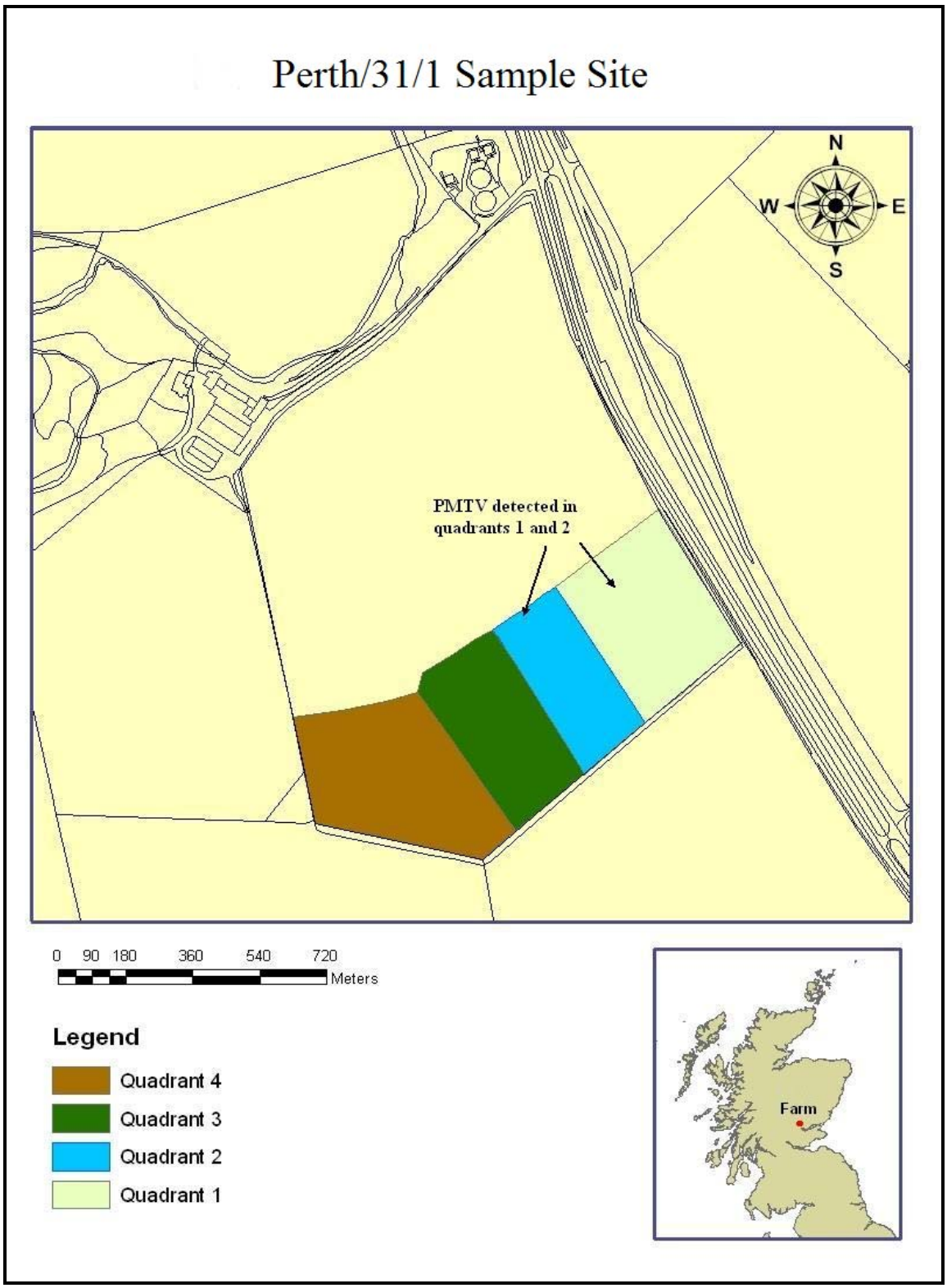

Figure 4-16. A field which produced a crop of cv. Cara with 52% infection in 2004 was sub-divided into four quadrants using a hand held GPS receiver. Soil was sampled in spring 2005 and tested for PMTV using a tomato bioassay and real-time PCR. Arrows indicate parts of the field in which PMTV was detected.

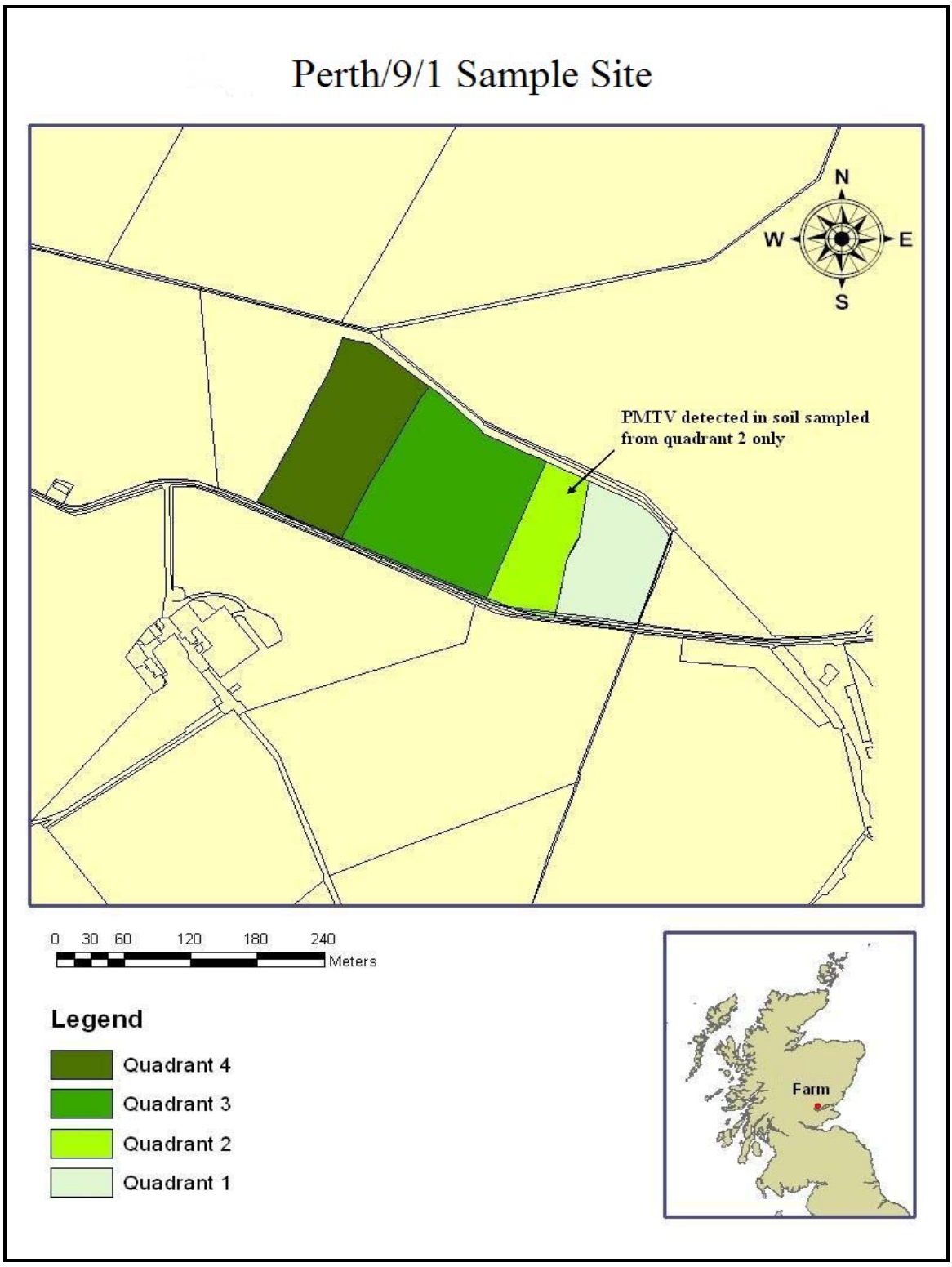

Figure 4-17. A field which produced a crop of cv. Cara with 35% infection in 2004 was sub-divided into four quadrants using a hand held GPS receiver. Soil was sampled in spring 2005 and tested for PMTV using a tomato bioassay and real-time PCR. Arrows indicate parts of the field in which PMTV was detected.

4.3.2.3 2006 soil samples

Prior to planting and after harvest, soil was sampled from the 28 fields in which class E1 seed potato crops of cv. Cara were grown (as described in Section 4.2.2.5.1). The aim was to determine whether infection of daughter tubers was related to the presence of PMTV inoculum in soil and whether PMTV was introduced into PMTV-free fields by planting infected seed tubers. In total, 56 soil samples were collected from the 28 fields.

4.3.2.3.1 Effectiveness of RNA 2 and RNA 3 primer/probe combinations in detecting PMTV in tomato plants grown in field soils

This section compares the effectiveness of the RNA 2 primer/probe combination with the RNA 3 primer/probe set of Mumford *et al.* (2000) in detecting PMTV in field soil. In total, 168 replicates were tested (3 replicates of each of the 56 soil samples). With the pre-planting soil samples, PMTV was not detected by either primer set in 120 (71%) of the replicates (Table 4-18). PMTV was detected in 30 (18%) replicates using both sets of primers and in a further 18 (11%) only with the RNA 2B primers.

In the post harvest soil samples, PMTV RNA was not detected in 96 (57%) of the replicates when tested using either primer set and 58 (35%) of the replicates were positive with both primer sets. The remaining 14 (8%) replicates were positive when tested using the primer set which targets RNA 2 but negative when tested for RNA 3. Overall, there was agreement between the two primer sets in detecting PMTV in pre-planting and post harvest samples (K = 0.7 and 0.8 respectively). However, in general, for both pre-planting and post harvest soil samples, RNA 2 was detected in a higher number of replicates than RNA 3, with the RNA 3 primers failing to detect PMTV in 37.5% of pre-planting and 20% of post harvest soils in which PMTV RNA 2 was detected.

Table 4-18. Number of soil sample replicates in which PMTV was detected in pre-planting and post harvest soils using primer sets RNA 2B and RNA 3B.

			RNA 3B			
			Positive	Negative	Total	K
Pre-planting	**RNA 2B**	Positive	30	18	48	**0.704**
		Negative	0	120	120	
		Total	30	138	168	
Post harvest		Positive	58	14	72	**0.826**
		Negative	0	96	96	
		Total	58	110	168	

The mean Ct values for the four soils in which PMTV was detected in all replicates of pre-planting or post harvest soils using both primer sets are shown in Table 4-19. As with the results of the 2004 study, the Ct values obtained using the RNA 2B primers were significantly lower than those obtained using the published RNA 3 primers for both pre-planting and post harvest soils (Table 4-19).

Table 4-19. Mean Ct values for PMTV RNA obtained by Real-time PCR for tomato bait plants grown in samples from 4 fields in which PMTV was detected in every replicate using both RNA 2 and RNA 3 primer sets.

	Pre-planting		**Post harvest**	
County/Grower/Farm	**RNA 2**	**RNA 3**	**RNA 2**	**RNA 3**
Perth/9/1	18.65	22.34	20.15	22.79
Perth/19/3	20.83	21.71	17.21	18.86
Angus/7/2	16.41	19.63	19.56	21.20
Angus/7/2	17.64	21.11	16.17	17.90
t-Test: Paired Two Sample for Means	**t (3 d.f.) = -4.3, P < 0.05**		**t (3 d.f.) = (-7.9), P<0.005**	

4.3.2.3.2 Relationship between detection of PMTV in pre-planting and post harvest samples of field soils and daughter tuber infection in crop

Because the RNA 2 primer/probe combination was more effective than the RNA 3 primer/probe combination in detecting PMTV in soil samples in 2004 and 2005, only the results for this primer set are presented. The occurrence of PMTV in both pre-planting and post harvest soil samples for each field, as expressed by the percentage of replicates in

which PMTV was detected, using RNA 2 primers, is shown in Table 4-20 together with incidence of tuber infection in each crop.

Table 4-20. Occurrence of PMTV in 28 field soils in which seed crops of cv. Cara were grown in 2006 as assessed by a tomato bioassay and real-time PCR using the RNA 2B primer/probe combination.

Area/Grower/ Farm	% PMTV in seed tubers	% PMTV in 2006 crop	Pre planting	Post harvest
Fife/23/5	0	0	*0/12 (0)	3/12 (25)
Fife/23/1	0	0.67	1/3 (33.3)	2/3 (66.67)
Angus/22/2	0	0	0/3 (0)	0/3 (0)
Angus/22/1	0	0	3/3 (100)	1/3 (33.3)
Angus/22/3	0	0.67	0/3 (0)	0/3 (0)
Angus/17/1	0	0	2/6 (33.3)	0/6 (0)
Angus/17/4	0	0	2/3 (66.7)	0/3 (0)
Angus/14/3	2	4.45	7/18 (38.9)	6/18 (33.3)
Angus/14/4	2	2.67	0/6 (0)	5/6 (83.3)
Angus/6/2	13.33	4.67	4/15 (26.7)	14/15 (93.3)
Angus/6/3	13.33	0.67	0/12 (0)	2/12 (16.7)
Perth/9/6	0	1.33	2/6 (33.3)	2/6 (33.3)
Perth/9/5	0	1.33	1/3 (33.3)	2/3 (66.7)
Perth/9/1	0	2.67	3/3 (100)	3/3 (100)
Perth/19/3	0	45.33	3/3 (100)	3/3 (100)
Perth/19/4	0	5.33	2/6 (33.3)	4/6 (66.7)
Aberdeen/29/2	0.67	3.33	2/6 (33.3)	2/6 (33.3)
Angus/25/2	12.7	1.33	1/6 (16.7)	1/6 (16.7)
Perth/31/3	9.33	8.67	2/3 (66.7)	3/3 (100)
Angus/30/2	1.33	0	0/6 (0)	2/6 (33.3)
Angus/13/1	2.70	0	2/6 (33.3)	2/6 (33.3)
Angus/27/3	0	0.67	2/6 (33.3)	0/6 (0)
Banff/28/1	0	6.67	0/3 (0)	3/3 (100)
Aberdeen/21/1	0	0	0/3 (0)	0/3 (0)
Angus/7/2	0	30.44	6/6 (100)	6/6 (100)
Perth/3/1	0.67	0.34	0/9 (0)	3/9 (33.3)
Perth/3/2	0.67	0	0/3 (0)	0/3 (0)
Angus/11/4	0.67	0	3/6 (50)	3/6 (50)

* The numerator is the number of replicates of field soil in which PMTV was detected; the denominator is the total number of replicates of field soil tested. The figure in parenthesis is the overall percentage of replicates of field soil in which PMTV was detected

PMTV was detected in 18 of the fields when sampled before planting and in 21 fields after harvest (Table 4-21). Of the 18 fields in which PMTV was detected before planting, the virus was recovered from samples taken from 15 of the fields after harvest. PMTV was found in post harvest samples from 6 out of the 10 fields in which PMTV had not been found before planting and, of these 6, PMTV had been detected in the seed potatoes used to plant four of these crops. The incidence of tuber infection in the seed potatoes ranged from 0.7 to 13.3 %.

Table 4-21. Number of fields in which PMTV was detected before and after planting by PCR assay using RNA 2 primer set.

		Pre-planting		
		PMTV-infested	PMTV-free	Total
After harvest	PMTV-infested	15	6	21
	PMTV-free	3	4	7
	Total	18	10	28

A summary of the results for soil sampled before planting and after harvest is presented in Table 4-22. Of the 5 crops produced in soil free of PMTV and from PMTV-free seed potatoes, two contained daughter tubers infected by PMTV. Post harvest soil samples from the two fields were also found to be infested by PMTV; however, only one of these fields was used to cultivate a crop in which PMTV was detected. Of the 5 crops derived from infected seed and planted in un-infested soil, 3 crops had tubers infected by PMTV and PMTV was detected after harvest in 4 fields. Planting PMTV-free seed in infested soil resulted in 8 out of 11 crops being infected, with 2 of these crops being the most infected, (Angus/7/2 - 30% and Perth/19/3 - 45%). However, only 8 out of the 11 infested fields were found to be infested when sampled after harvest. Planting PMTV-infected seed in infested soil resulted in PMTV infected daughter tubers in 5 out of 7 crops. All fields which were infested prior to planting were also infested following harvest.

Table 4-22. Proportion of crops with infected daughter tubers and field soils infested by PMTV after harvest in relation to PMTV-health of seed and infestation of soil pre-planting.

Health of seed potatoes	Pre-planting soil	Crops with infected daughter tubers	Post harvest soils infested
PMTV-free	PMTV-free	2/5	2/5
PMTV-free	Infested	8/11	8/11
Infected	PMTV-free	3/5	4/5
Infected	Infested	5/7	7/7

4.3.3 Temperature and PMTV infection from soil inoculum

An experiment was conducted in glasshouses to assess the effect of temperature on infection in plants and tubers of five cultivars produced in soil known to be infested by PMTV. The temperatures were 12, 19 and 26°C and the cultivars were Cara, Nicola, Rooster, Saturna and Slaney. PMTV-free seed tubers, as determined by ELISA, were planted in either John Innes No. 2 or a 50:50 mixture of John Innes No. 2 and a PMTV infested field soil. Symptoms of PMTV infection were not observed on the foliage, although some plants of cvs Cara, Nicola, Rooster and Saturna showed symptoms of infection by other viruses. In these cases, leaf testing by ELISA identified PVY^{N}; a tuber-borne, aphid transmitted potyvirus (data not shown).

PMTV was not detected in any daughter tubers produced at 26°C (Figure 4-18). The incidence of PMTV infection in tubers produced at 12° and 19°C in infested soil did not differ significantly ($P = 0.476$), with a mean incidence of 74 and 73% respectively; however, differences in cultivar susceptibility to PMTV infection were observed at 12°C ($P = 0.026$) but not at 19°C ($P = 0.117$). The highest incidence of PMTV infection (87.50%) was detected in daughter tubers of cv. Nicola grown at 12°C in infested soil; however, tubers of cvs Cara and Rooster produced at 19°C contained comparably high amounts of PMTV (85.19% and 86.36% respectively). With tubers produced in compost, a low amount of infection was detected in cvs Cara and Rooster at 12°C and in cvs Nicola and Saturna grown at 19°C.

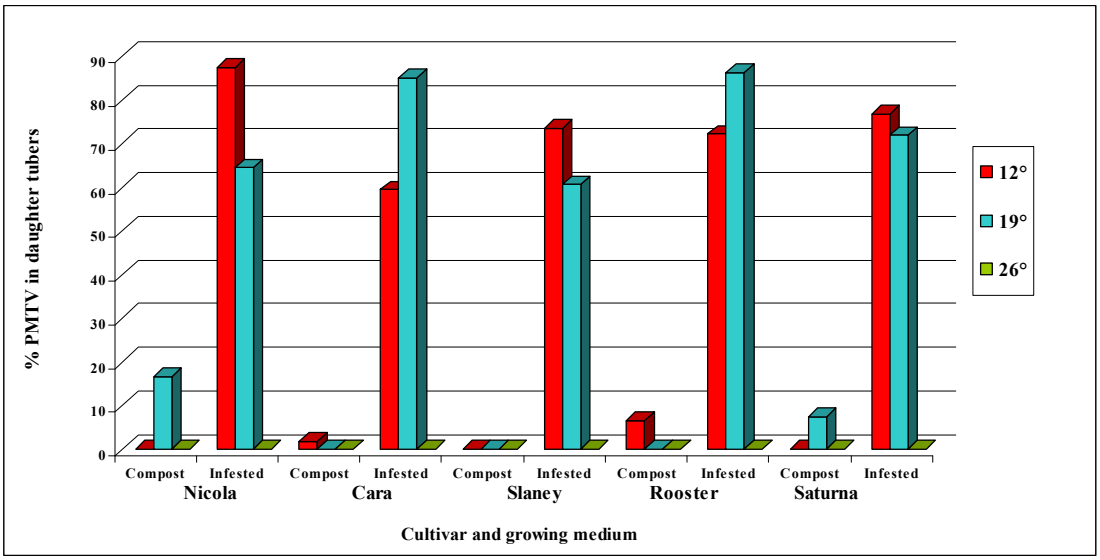

Figure 4-18. Incidence of PMTV in daughter tubers derived from PMTV-free seed tubers cultivated in either compost or soil known to be infested with PMTV.

Spraing only developed in daughter tubers produced at 12°C (Table 4-23) (see Figures 4-19 - 4-22 for examples of spraing symptoms in tubers from this study). There were clear varietal differences in the development of spraing symptoms. Cultivars Nicola and Cara were most sensitive to infection, with 36.1% and 28.6% of daughter tubers affected respectively. Cultivar Saturna was the least sensitive to tuber infection by PMTV, with only 2.6% of tubers developing spraing symptoms. Spraing symptoms were also observed in daughter tubers of cvs Cara and Rooster grown in compost (3.8% and 6.7% respectively); PMTV infection was confirmed by ELISA. The mean incidence of spraing in tubers was weakly correlated ($r = 0.58$, 5 d.f.) with the mean score for severity of spraing.

Table 4-23. The incidence of PMTV and spraing symptoms in daughter tubers grown in a 12°C glasshouse. The spraing severity score is also shown for each cultivar.

Variety	Treatment	% PMTV in daughter tubers	% daughter tubers with spraing symptoms	Spraing Severity Score (1-3)
Nicola	Compost	0.0	0.0	0.0
Nicola	Infested	87.5	36.1	2.7
Cara	Compost	1.9	3.8	1.5
Cara	Infested	59.5	28.6	2.7
Slaney	Compost	0.0	0.0	0.0
Slaney	Infested	73.6	18.9	1.8
Rooster	Compost	6.7	6.7	1.5
Rooster	Infested	72.2	7.4	2.3
Saturna	Compost	0.0	0.0	0.0
Saturna	Infested	76.9	2.6	1.0

Figure 4-19. External spraing symptoms on a tuber of cv. Nicola. Tubers were grown in an infested soil mixture.

Figure 4-20. Spraing symptoms emanating from the stolon end of a tuber of cv. Cara. Tubers were grown in an infested soil mixture.

Figure 4-21. Severe external spraing symptoms on a tuber of cv. Cara. Tubers were grown in an infested soil mixture.

Figure 4-22. Moderate external spraing symptoms on a tuber of cv. Slaney. Tubers were grown in an infested soil mixture.

Powdery scab lesions were not observed on tubers harvested from the 26°C glasshouse so only the results for 12°C and 19°C treatments are presented in Figure 4-23. Examples of powdery scab lesions on tubers of cvs Rooster, Saturna, Slaney, and Cara are shown in Figures 4-24 to 4-27 respectively. Significantly more of the tubers produced in the 12°C glasshouse were affected by powdery scab than those grown in the 19°C glasshouse ($P < 0.001$). Cultivar Saturna was badly affected with powdery scab; lesions were observed on 84.6% of daughter tubers grown at 12°C and 28% grown at 19°C. Powdery scab lesions were also observed on daughter tubers of cvs Cara and Rooster grown in compost. PMTV and spraing were also detected at 1.9 and 6.7%, and 3.8 and 6.7% respectively.

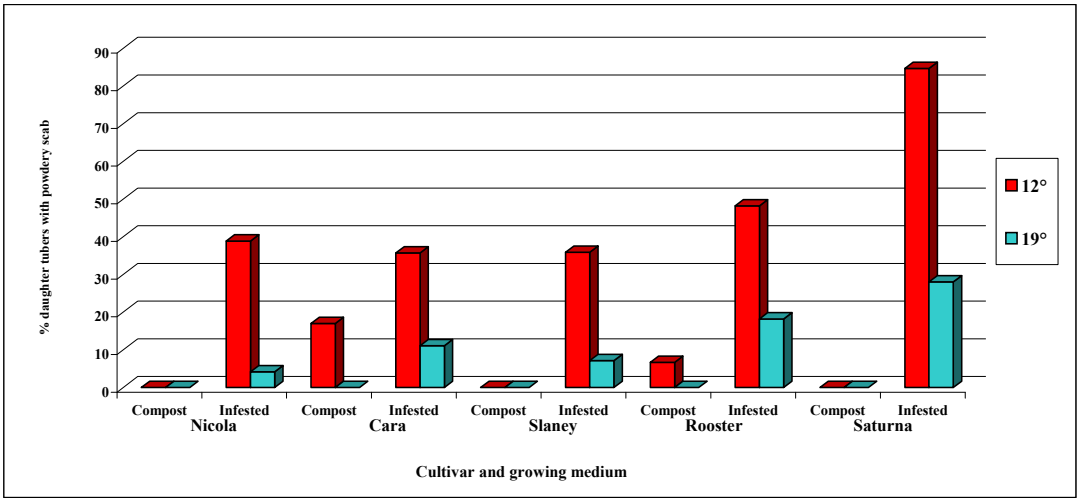

Figure 4-23. Incidence of tubers affected by powdery scab. PMTV- free seed tubers were cultivated in either compost or soil known to be infested with PMTV.

Table 4-24 shows the mean percentage of daughter tubers affected by powdery scab when plants were grown in a glasshouse at 12°C or 19°C. The powdery scab severity score was calculated using the scoring table in Section 4.2.2.6.3 to give an overall indication of tuber infection on affected tubers only.

Table 4-24. Mean incidence and severity score of powdery scab for all cultivars grown at 12°C and 19°C.

		12°C glasshouse		19°C glasshouse	
Treatment	**Cultivar**	**% Powdery Scab**	**Severity Score (1-75)**	**% Powdery Scab**	**Severity Score (1-75)**
Compost	Nicola	0.0	0.0	0.0	0.0
Infested	Nicola	38.9	15.4	4.12	1.0
Compost	Cara	17.0	13.5	0.0	0.0
Infested	Cara	35.7	4.2	11.1	1.0
Compost	Slaney	0.0	0.0	0.0	0.0
Infested	Slaney	35.9	2.9	7.1	1.0
Compost	Rooster	6.7	4.3	0.0	0.0
Infested	Rooster	48.2	11.3	18.2	1.7
Compost	Saturna	0.0	0.0	0.0	0.0
Infested	Saturna	84.6	18.0	28.0	1.0

There was a weak correlation between the mean incidence of powdery scab at both 12°C (r=0.58, 5 d.f.) and 19°C (r=0.26, 3 d.f.) and the severity of the disease. At 12°C, cv. Rooster was more susceptible to powdery scab than cv. Nicola (48.15% and 38.9% respectively), though cv. Nicola had a higher severity score. Powdery scab lesions were observed on tubers of some cultivars grown in compost at 12°C but not at 19°C. For example, a moderate incidence of powdery scab was observed on daughter tubers of cv. Cara grown in compost at 12°C (17%); this was significantly lower than the incidence occurring with the compost/infested soil mixture. The severity scores however, indicate that the tubers of cv. Cara grown in compost were significantly more severely affected by powdery scab lesions than those grown in the infested soil (score 13.5 and 4.2 respectively).

Figure 4-24. Powdery scab lesions on cv. Rooster. Tubers were grown in an infested soil mixture.

Figure 4-25. Powdery scab lesions on cv. Saturna, the most susceptible cultivar in the study. Tubers were grown in an infested soil mixture.

Figure 4-26. Powdery scab lesions on cv. Slaney. Tubers were grown in an infested soil mixture.

Figure 4-27. Powdery scab lesions on cv. Cara. Tubers were grown in an infested soil mixture.

At 12°C, cv. Nicola had the highest yield of the five cultivars grown in compost and infested field soil at 0.90 and 0.52kg per plant respectively and cv. Saturna had the lowest yield of daughter tubers derived from healthy seed tubers planted in compost and infested soil. Cultivar Nicola was most affected by planting in infested soil with a decrease in yield of 0.38kg per plant. Cultivar Rooster was least affected by planting in infested soil with a decrease of 0.17kg per plant.

The yields obtained at 19°C were broadly similar to those obtained at 12°C (P = 0.026). Again, cv. Nicola had the highest yield of the five cultivars grown in both compost and infested field soil at 0.81kg and 0.48kg respectively and cv. Saturna had the lowest yield of daughter tubers derived from healthy seed tubers planted in compost (0.43kg per plant); however cv. Cara had the lowest yield per plant at 0.24kg when seed tubers were grown in infested soil. As in the 12°C glasshouse, cv. Nicola was most affected by planting in infested soil with a decrease in yield of 0.32kg per plant and cv. Saturna was least affected by planting in infested soil with a decrease of only 0.17kg per plant.

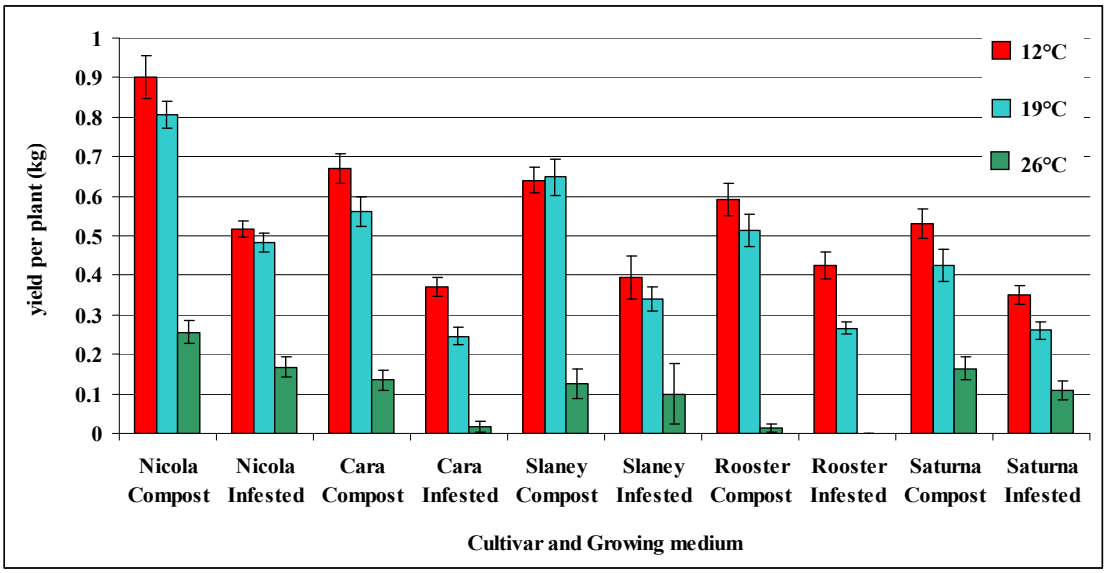

Figure 4-28. Mean yield/plant (kg) for cvs Cara, Nicola, Rooster, Saturna and Slaney grown in either compost or infested field soil at three different temperatures of 12°C, 19°C or 26°C.

Seed tubers grown at 26°C produced a significantly smaller yield. All 5 cultivars were affected by the high temperature, however there were differences in the yield per plant amongst the cultivars tested ($P< 0.001$). As described for the yield of daughter tubers grown at both 12°C and 19°C, cv. Nicola had the highest yield of the 5 cultivars grown in both compost and infested field soil at 0.26kg and 0.18kg respectively. Cultivar Rooster had the lowest yield of daughter tubers derived from healthy seed tubers planted in compost (0.01kg per plant) and also did not yield any daughter tubers from the infested soil. Rooster was the only cultivar that did not yield any daughter tubers at this high temperature. Cultivar Cara was most affected by planting in infested soil with a decrease in yield of 0.12kg per plant and, aside from cv. Rooster, cv. Slaney was least affected by planting in infested soil with a decrease of 0.03kg per plant.

4.4 Discussion

4.4.1 Methodological development

4.4.1.1 Developing an effective soil bioassay

To further investigate and clarify the importance of soil inoculum in causing PMTV outbreaks in a crop, bioassays were performed on all soils used to cultivate crops of cv. Cara in 2004 and 2006. In 2006, pre-planting and post harvest soils were tested for the presence of PMTV in order to establish whether the level of pre-planting soil inoculum could be used as a predictor of infection. Soil testing also allowed for a direct comparison between inoculum levels in the fields prior to planting the crop and after harvesting the crop.

The bioassay developed for use on these soil samples generally proved to be very effective in detecting PMTV and when used in conjunction with real-time RT-PCR, results could be generated more rapidly than previously described bioassays (Kurppa, 1989a, Arif *et al*., 1994). In the study by Kurppa (1989a), bait plants of both *Nicotiana clevelandii* and *N. debneyi*, were grown for eight to ten weeks in the sample soil prior to inoculating leaves of indicator plants of *Chenopodium quinoa* with infective sap from the ground root tissue of the bait plants. The indicator plants were then grown for a further three to four weeks. Although this method was effective in detecting the virus in infested field soils, the process was time consuming, taking more than 11 weeks to obtain a result. Arif *et al.* (1994) found that using *N. debneyi* as a bait plant in conjunction with RT-PCR could be used effectively to detect PMTV in the roots and leaves after three weeks. The combined use of *L. esculentum* cv. Moneymaker as a bait plant and real-time RT-PCR in the bioassay described in this chapter, was effective in further reducing the time taken to obtain results, with detection possible after two weeks.

The value of an effective and rapid soil bioassay for PMTV is clearly demonstrated by the findings presented in Tables 4-14 and 4-22, which show the link between soil inoculum and PMTV infection in a crop. It is encouraging that with crops Perth/31/1, Perth/9/1, Angus/5/1, Perth/19/3 and Angus/7/2, all soil replicates tested positive for PMTV, as cultivation in these soils resulted in very high levels of infection in the daughter tubers

when grown from seed potatoes containing no or minimal amounts of PMTV infection. Furthermore, field soils generally tested negative where the crop was also found to be free of PMTV by post harvest tuber testing. The soil bioassay therefore clearly offers the potential for determining which fields contain soil inoculum and are likely to result in PMTV infected daughter tubers if planted.

This assay also detected PMTV in field soils that produced crops with low levels or a complete absence of infection in the daughter tubers, even in some fields in which all soil replicates tested positive for PMTV. For example, PMTV was detected in all replicates of soil from a field used to cultivate crop Angus/27/1; however only 0.5% of the tubers from this stock were found to be infected with PMTV. It is not known why some field soils that yielded cv. Cara stocks with low levels of infection produced similar results/comparable Ct values to soils yielding highly-infected stocks. It may be that the sampling strategies in place for sampling soil-borne pathogens are not appropriate for PMTV and an alternative sampling regime may need to be developed for this pathogen. It may also be that the variability in environmental factors across Scotland/between fields plays a part, with PMTV-carrying *S. subterranea* present in the soil of certain fields, but environmental conditions or poor drainage enhancing the level of PMTV transmission and ultimately, infection, between/in different fields. Cooper and Harrison (1973), for example, reported that the probability of PMTV infection increases with an increase in annual rainfall, while Kirk (2008) showed that high soil moisture levels during tuber initiation increase the risk of infection. Overall, it is clear that while the bioassay represents a useful tool for qualitatively assessing the risk of PMTV infection in any one field, it cannot quantitatively assess risk.

The results from the bioassay were difficult to interpret on occasion, due to a lack of consistency between replicates within each soil sample. For example, PMTV was only detected in one replicate of soil in which crop Angus/30/2 (2.5% PMTV infection) was grown. Such inconsistency may potentially result in difficulties in giving growers definitive advice. It is possible that these discrepancies can be attributed to sampling problems, either in obtaining a representative sample of tubers from the crop or the field soil, or even the efficiency of the bioassay itself. As Brierley *et al*., (2009) noted, it is critical that field soil sampling strategies should be designed to be representative of the

whole field. In order to overcome discrepancies in results, the bioassay method should be further refined; either by bulk extracting RNA from all seedlings grown in the sample soil, thereby giving one result per field soil sample, or by increasing the number of sampling points within a field, thereby increasing the likelihood of detecting PMTV inoculum.

The lack of consistency within replicates of sample soil also indicates that PMTV may be discontinuously distributed in a field and there may be 'hotspots' where PMTV-carrying *S. subterranea* are established. These 'hotspots' may be attributed to variability in environmental factors at the field scale. Soil moisture, highlighted by Kirk (2008) as a key influence on the development of PMTV infection, is likely to be affected locally by factors such as gradient and soil compaction. This apparently non-uniform distribution of PMTV within fields would appear to be confirmed by the results presented in Section 4.3.2.2.1, where PMTV inoculum was found to be unevenly distributed in two sample field sites. These sites were chosen due to the high incidence of PMTV infection (35% and 52%) detected in tubers grown there. In an earlier study of PMTV distribution within fields, Jones and Harrison (1972) collected six soils from a number of sites within one field and tested the samples for PMTV using bait plants; all the soil samples differed widely in their infectivity. Soil samples collected near gates or walls were particularly infective and the amount of PMTV inoculum was shown to vary widely between sampling points within close proximity to each other. It may be that soil at gates and field boundaries is more compacted and less free draining, leading to a build up of inoculum at these sites.

In order to investigate and determine the distribution of PMTV inoculum within fields, further study is required. Specifically, fields could be split further into smaller subunits for sampling or different sampling strategies could be evaluated, which may enable a sampling strategy to be developed specifically for the detection of PMTV. However, this may be impractical due to the increase in time required to sample fields, especially if a field is to be tested for a number of soil-borne pathogens that require different sampling techniques.

4.4.1.2 Effectiveness of RNA 2 and RNA 3 primer/probe combinations in detecting PMTV

Five new real-time primer/probe combinations were designed in order to compare their specificity and sensitivity with a set of primers designed by Mumford *et al.* (2000) and potentially broaden the options for molecular detection of PMTV. PMTV particles contain three single stranded positive sense RNA molecules (Kallender *et al*., 1990; Scott, 1994). Two real-time primer sets were designed for each RNA molecule in the PMTV genome; however, only one was designed to target RNA 3, as the primer set designed by Mumford *et al.,* (2000) amplifies a sequence of the RNA 3 molecule that encodes the coat protein.

PMTV RNA was extracted from infected tuber tissue and screened using all six primer sets. Of these six primer/probe combinations, the newly designed RNA 2B and RNA 3B (Mumford *et al*., 2000) were found to be the most sensitive. The RNA 2B primer set amplified a sequence within protein 3 of the triple gene block proteins, involved in cell to cell movement (Haupt *et al*., 2005).

Comparison of the 2 primer sets did show a high degree of correlation. However, in 8% of cases, the RNA 2B primers detected PMTV when the RNA 3B primers did not. Furthermore, PMTV RNA 2 was detected in all samples in which PMTV RNA 3 was detected, minimising the risk of reporting false negative results. These results also indicate that PMTV RNA 2 may be present in greater quantities in PMTV infected tissue, or may amplify more efficiently than RNA 3, as illustrated by significantly lower Ct values.

The coat protein is a logical target for a detection assay as it is a highly conserved region of the PMTV genome (Reavy *et al*., 1997), and this approach has proven to be an effective method of detecting PMTV. However, not all plant parts infected with viral RNA necessarily contain assembled virions (Xu *et al*., 2004). As McGeachy and Barker (2000) reported, systemic movement of PMTV is not dependent on the presence of coat protein expression or virion formation. The triple gene block proteins, which are encoded by RNA 2, represent a class of long distance RNA movement factors that mobilise naked, but infectious RNA. The result being that naked PMTV RNA may be present in symptomless tubers and cause secondary infection the following season (Xu *et al*., 2004). Furthermore,

the coat protein has been shown to be involved in symptom expression (McGeachy and Barker 2000). As symptom expression of PMTV infection is variable in both plants and tubers, it may be that assembled virions are not present, in which case, targeting RNA 2 may be a more effective approach for the detection of PMTV.

Detection methods based on RNA 2 have not been reported previously and the results shown here indicate that PMTV RNA 2 is an effective target for molecular detection.

4.4.2 The importance of seed inoculum on PMTV infection

The seed production programme for cv. Cara gave a unique opportunity to examine the transmission and occurrence of PMTV, spraing and powdery scab between two consecutive seed generations of tubers, when crops of known provenance were grown over two years at a range of sites in Scotland. As the incidence of PMTV infection in the seed tubers was known, this allowed inferences to be made about possible sources of infection in the daughter crops and their relative importance. The results of this study were consistent over the two multiplication cycles, adding further weight to these findings.

The overall incidence of PMTV in seed tubers from the initial producer was very low in both years. After multiplication for one year by basic seed potato producers, most crops were still relatively free from PMTV; however, each year, a number of crops with large incidences of PMTV infection were recorded. However, these anomalies did not appear to be linked to initial amount of infection in the seed potatoes, with no correlation evident overall, between the incidence of PMTV in seed potatoes and that in daughter crops. In a number of cases, the difference between the incidence of PMTV infection in mother and in daughter crop was particularly pronounced; for example, when seven crops of cv. Cara from 2005 and 2006, were grown from seed tubers (Angus/5/1, Perth/9/1, Angus 6/2 and Angus/25/2) with 12-35% tuber infection, the incidence of PMTV infection in the daughter crops was considerably less, ranging from 0.7 - 4.7% infection and the reduction in PMTV infection varied between 65 - 95%. This is greater than the 55% reduction reported when daughter tubers of cv. Cara were derived from infected seed tubers (described in Section 3.3.1.3); however, this difference in elimination of PMTV infection may be attributable to the lower incidence of PMTV infection in these seed tubers compared to 100% infection in the cv. Cara seed transmission experiments described in Chapter 3. Nevertheless, this

elimination of PMTV infection (combined with the occasional high increase in infection in a small number of cases) confirms the findings reported by Calvert (1968), Cooper *et al*., (1976) and the findings reported in Chapter 3, namely that in the absence of infection from soil inoculum, PMTV will be greatly reduced from a crop within one or two multiplications. These results provide clear evidence that soil inoculum rather than seed inoculum is likely to be the major factor causing serious infections of potato crops.

4.4.2.1 The relationship between PMTV infection, powdery scab and spraing symptoms

In contrast to the predominantly low incidence of PMTV in seed potato crops, powdery scab was widespread on all stocks of cv. Cara across all three years of this study, indicating that either not all populations of *S. subterranea* carry PMTV or that the conditions required for infection are different for both pathogens. The former hypothesis is supported by Jones and Harrison (1969), whereby host plants of *Nicotiana debneyi,* grown in soil artificially inoculated with *S. subterranea* sporeballs, consistently failed to develop symptoms of PMTV infection, whereas *N. debneyi* became infected when scrapings were taken from alternative stocks in which PMTV symptoms were obvious in the tubers.

Overall, no correlation was found between the incidence of powdery scab and the incidence of PMTV infection in daughter tubers. This finding agrees with previous findings by Nielsen and Nicolaisen (2000); Tenorio *et al.* (2006) and Montero-Astúa *et al.* (2008). It is interesting to note that Montero-Astúa *et al.* (2008) detected PMTV infection in the absence of *S. subterranea* in 18% of paired leaf/tuber samples tested by ELISA. Conversely, they also detected *S. subterranea* in 13% of samples in which PMTV was not detected. As PMTV is generally considered to be found with its vector (Calvert and Harrison, 1966), the absence of *S. subterranea* in PMTV-infected tubers is surprising. However, this can most likely be explained by the method of detection used in their study. The antibodies used in their ELISA testing were specific to resting spores of *S. subterranea*, which meant they effectively ignored the other stages of the pathogen's life cycle and could underestimate the level of infection.

Tenorio *et al.* (2006) also found no correlation between the incidence of PMTV infection, as determined by ELISA on nitrocellulose membrane (NCM-ELISA), and the incidence

and severity of powdery scab lesions on 21 U.S. cultivars grown at three different sites in the Peruvian Andes. For example, powdery scab was most severe on tubers of cv. Gold Rush; however, this cultivar was amongst those least susceptible to PMTV infection. These findings also reflect the current powdery scab situation in Denmark, where powdery scab lesions are rarely seen on PMTV infected tubers despite *S. subterranea* being present. As Nielsen and Nicolaisen (2000) noted, *S. subterranea* is, in fact, a greater problem for the Danish potato processing industry in terms of its capacity to act as a vector for PMTV, which frequently results in economically significant incidences of spraing in that country, especially in cv. Saturna (Nielsen and Mølgaard, 1997), than it is as a pathogen in its own right.

In general, powdery scab infection is more widespread than PMTV - in this and previous studies, although it is often the case that lesions are not obvious during visual examination. For example, in all three years of data presented here, PMTV was detected in a low number of crops in which powdery scab was not observed on tubers. However, in all cases, the incidence of PMTV infected tubers in the crops was low; the highest being 2%. It is likely that powdery scab sporeballs may have been present on the tubers from these crops; however, the lesions may not have been visible. In this case, testing tubers for *S. subterranea* by ELISA or real-time PCR would allow for a more definitive diagnosis. The absence of powdery scab lesions on PMTV-infected tubers may be explained, in part, by environmental conditions. It may be that the environmental factors which favour the development of powdery scab lesions differ from those required for successful transmission of the virus to the host by its vector or it may be that the main route of transmission of PMTV is through the root tissue of the host plant. This may explain, in part, the detection and/or the prevalence of the symptoms of either pathogen at any one time.

A good correlation was observed between PMTV infection and the occurrence of spraing in crops, indicating that for cv. Cara, as with cv. Saturna (as discussed in Chapter 2), spraing symptoms may be a good indicator of PMTV infection (Kurppa, 1989a). The occurrence of spraing is a symptom of primary infection (Calvert and Harrison, 1966), i.e., infection from the soil in the year of planting, indicating that, where spraing is observed in a crop, the soil in which the crop is grown is infested with PMTV-carrying *S. subterranea*. This further highlights the importance of soil inoculum in causing outbreaks of PMTV.

Overall, the lack of any significant relationship between the PMTV health of the seed tubers (i.e., PMTV infection, the occurrence of spraing and the occurrence of powdery scab) and the health of daughter tubers across the three years of this study conclusively indicates that the health of seed tubers is not the major factor influencing the incidence of PMTV in daughter tubers. The role of soil inoculum is clearly the most significant factor.

4.4.3 The importance of soil inoculum on PMTV infection

Overall, the findings of the 2006 study, which involved both pre-planting and post harvest soil bioassays, confirm the importance of soil inoculum in causing infection. Planting PMTV-free seed in PMTV-infested soil resulted in 8 out of 11 crops yielding PMTV-infected daughter tubers. However, planting infected seed tubers in PMTV-free soil resulted in 3 out of 5 crops producing PMTV-infected daughter tubers. It is concerning that 4 out of 5 soils became infested by PMTV when sampled post harvest after planting infected seed in PMTV-free soil. This suggests that planting tubers carrying PMTV may be spreading the pathogen within Scotland and that uninfested fields may be contaminated in this way.

It is evident that, while PMTV may be self-eliminating over time in a stock (see Chapter 3), this is only likely to be the case when seed is planted in PMTV-free soil. As such, soil inoculum is the major cause of PMTV infection. Furthermore, PMTV remains infective in soil for long periods of time, with Calvert (1968) reporting PMTV infected tubers produced in a field 18 years after the last potato crop highlighting the persistent nature of this problem. The control of PMTV in Scotland is clearly made more complex by the finding that infection can be spread and established at new sites by planting infected seed tubers. It may be unrealistic to expect growers not to plant infected tubers in uninfested sites, or to avoid planting healthy tubers in infested sites; as such, the best approach may be to use integrated control measures for the control of PMTV. The avoidance, where possible, of infested field soils and infected seed is clearly important; however, the use of more tolerant cultivars is also likely to play a key role in minimising the economic effects of PMTV on potato crops. For example, cvs Maris Piper and Desiree have both been shown to be tolerant to PMTV infection in a study by Carnegie *et al*, (2009), with no spraing observed in tubers of these cultivars despite a high incidence of PMTV infection being detected (63%

Maris Piper and 73% Desiree). The use of these tolerant cultivars in potato growing regions of Scotland which are known to be infested with PMTV (see Chapter 2) is to be particularly recommended.

To date, a tolerance for PMTV has not been included in the Scottish Seed Potato Classification Scheme, mainly due to studies which have shown the virus to be self-eliminating (Cooper *et al.*, 1976). However, some countries, for example Brazil, on the basis of Pest Risk Analysis, stipulate a nil tolerance for PMTV and tuber symptoms. Strict import requirements could have a detrimental effect on potential Scottish export trade in seed potatoes if adequate control measures are not implemented. A detailed study is, therefore, required to determine the most effective control measures and whether a tolerance for PMTV should be included in the classification scheme.

4.4.4 Temperature and PMTV infection from soil inoculum

Environmental factors influencing infection by PMTV are likely to be linked to those favoured by its vector, *S. subterranea* (see Section 1.3.1.1). The results presented here, show that both the incidence and severity of powdery scab is likely to be greater on daughter tubers produced at 12°C than at 19°C, although severity of symptoms was not linked to incidence. These results support those previously reported by de Boer *et al.* (1985), van de Graaf *et al.* (2005) and Carnegie *et al.* (2009), who report a higher incidence of powdery scab on daughter tubers produced at 12°C than on those produced at higher temperatures of 17°C, 19°C or 20°C; symptoms were absent at 10°C in experiments conducted by de Boer *et al.* (1985); however, symptoms have been visually observed on tubers grown at 9°C in experiments by van de Graaf *et al.* (2005). It is apparent from the data presented in this study that both the incidence and severity of powdery scab decline with increasing temperature, with an absence of symptom development at 26°C. The first record of *S. subterranea* in Malta, for example, was associated with below normal temperatures in the autumn and winter of 2004-2005 (Porta-Puglia and Mifsud, 2006).

The findings shown here indicate that symptom development of *S. subterranea* infection is cultivar dependent; cv. Saturna is particularly susceptible to powdery scab at all temperatures. In Denmark, cv. Saturna is considered to be a cultivar which is highly susceptible to PMTV infection; however, powdery scab is not considered a serious problem

in Denmark, except in relation to a small number of cultivars e.g. Kennebec and Asparges (Nielsen and Nicolaisen, 2000). It may be the case that the mild climate in Denmark allows for infection by *S. subterranea* and PMTV but not powdery scab development.

The results shown here indicate a difference in the temperature range in which infection by PMTV and development of powdery scab lesions occurs. This may be due to transmission of PMTV from the vector *S. subterranea*, occurring across a wider temperature range than powdery scab symptoms. Differences in cultivar susceptibility to PMTV infection were observed at 12°C but not at 19°C; however, there was no significant difference in the incidence of infection (74% and 73% respectively). It is likely that 19°C is a more optimal temperature for transmission of PMTV from the vector because greater virus pressure at this temperature eliminated differences in susceptibility of the cultivars. This optimal temperature for PMTV infection is higher than the optimum temperature of 12-15°C reported for powdery scab (Merz, 2008). This may partly explain why PMTV has often been found in crops largely unaffected by powdery scab (Nielsen and Nicolaisen, 2000; Tenorio *et al*., 2006 and Montero-Astúa *et al*., 2008) and not in direct association with powdery scab lesions on tubers (Jones and Harrison, 1969).

The incidence of PMTV infection in this temperature study was higher than the incidence in the seed transmission study (Chapter 3) but similar to some of those in the cv. Cara study. Overall, the results of my studies suggest that high incidences of PMTV infection are more likely to be attributable to infection from soil inoculum than seed inoculum; therefore, immediate economic losses will be associated directly with infection through soil rather than the mother tuber. However, as the experiments in this study were conducted in controlled environments, these results may be exaggerated compared to what might be observed in field conditions. Tubers grown in a field are subjected to wide daily fluctuations in temperature and moisture, whereas the temperatures in the glasshouse studies were relatively constant.

Interestingly, PMTV infection, powdery scab and spraing symptoms were observed on some cultivars grown in compost at 12°C. The occurrence of spraing symptoms in these infected tubers indicates that the infection is a primary infection and was more than likely derived from the growing media. It may be that that contamination occurred during the

preparation of the infested soil:compost mixture. Indeed, Helias and Bourdin (2000) reported a similar finding from a study in France, whereby crops grown in a glasshouse on imported peat substrates were found to be infected with PMTV resulting in the entire crop having to be completely destroyed. It also remains a possibility that airborne particles of *S. subterranea* containing PMTV may have contaminated the compost filled pots. However, Jones and Harrison (1969) demonstrated that contamination of soils by airborne PMTV-carrying *S. subterranea* is unlikely. Specifically, this study involved drying infested and non-infested soils side by side on a bench and testing the bait plants that were subsequently planted in each of the soils. Therefore, it would appear more probable that the compost used in the control pots was infested prior to use.

As with powdery scab, spraing symptoms were also only observed in daughter tubers produced at 12°C and not 19°C or 26°C. This may explain why spraing has not been recorded in PMTV-infected tubers produced in countries with higher temperatures, such as Costa Rica (Montero-Astúa *et al*., 2008). Interestingly, the cultivar in this study that was most susceptible to powdery scab (cv. Saturna) was also the most tolerant to spraing symptoms. A previous study conducted in Scotland by Sokmen *et al*., (1998) also found cv. Saturna to be less susceptible to spraing. In contrast, spraing symptoms in cv. Saturna have been reported by Kirk (2008), as causing serious problems for potato production in Sweden. It is uncertain as to why these differences in susceptibility to spraing in cv. Saturna occur between countries; however, it may be due to differences in climatic factors or conditions of storage resulting in symptom expression.

Overall, the development of symptoms of both PMTV and powdery scab are temperature dependent, although transmission of PMTV infection is less so. As such, PMTV and its symptoms may not present itself as a serious problem for the Scottish seed potato export industry, as the majority of the seed potato export market is represented by countries with significantly warmer climates than Scotland (Scottish Government 2009b). Furthermore, climate change, where it results in increasing temperatures, may also result in a decline in the occurrence of tuber PMTV infection, although, this may be confounded by increased annual precipitation.

Chapter 5. Conclusions

A key objective of this work was to determine the distribution of PMTV throughout the main seed potato producing areas of Scotland. The results presented in Chapter 2 clearly demonstrate that PMTV is widespread in all four major seed potato growing regions; however, the actual incidence of infection in crops was found to be generally low. A greater proportion of susceptible potato cultivars were infected when grown in Central Scotland and a lesser proportion when grown in south-East Scotland. Furthermore, the results shown in Chapter 2 demonstrate that latent infection by PMTV is common in the four cultivars included in the study (cvs Hermes, Maris Piper, Nicola and Saturna).

The results presented in Chapter 3 demonstrate that not all plants grown from tubers known to be infected with PMTV develop foliar symptoms; however, some cultivars were more likely to express symptoms than others. Furthermore, asymptomatic foliar infection by PMTV was common in all cultivars studied. It was concluded that symptoms of PMTV infection in the growing plant are not a reliable indicator of infection and as a consequence roguing may have little impact on the removal of PMTV from a crop.

The results of the field trials presented in Chapter 3 and the survey of cv. Cara in Chapter 4 clearly show that transmission of PMTV from infected seed to daughter tubers is relatively inefficient, with less than 50% of daughter tubers becoming infected when grown from infected mother tubers. Furthermore, the findings from the cv. Cara survey (Chapter 4) show that the health of seed tubers, as measured by symptom expression and the occurrence of powdery scab, had no direct influence on PMTV infection of daughter tubers. These findings confirm that PMTV is self-eliminating and, in the absence of new infection, may be greatly reduced or eliminated from a stock within a few multiplication cycles.

The results in Chapter 4 showed clearly that, overall, inoculum from soil is of greater importance than from seed in causing significant outbreaks of PMTV and in subsequent potato crops. Planting PMTV infected tubers, is likely to facilitate the spread of the virus to new sites, however, further research is required to conclusively determine the risk. The soil bioassay developed in Chapter 4 could prove to be an effective tool in the formulation

of advice to growers on both site and cultivar selection. Specifically, soil testing could be used to ensure the successful avoidance of infested soils or highlight the need for a tolerant cultivar, i.e., one which does not express symptoms, when soil is known to be infested.

The temperature experiments in Chapter 4 show that temperature had a greater influence on the development of powdery scab lesions than on PMTV infection. Powdery scab development had an optimum of 12°C-15°C. The optimum temperature for the transmission of PMTV infection from infested soil was shown to range from 12°C to 19°C. In contrast, the expression of spraing symptoms in PMTV infected tubers was greatly influenced by temperature, with symptoms common at 12°C but absent at 19°C.

In summary, it is clear from the findings presented here that PMTV is dispersed throughout the potato growing regions of Scotland, although the actual incidence of PMTV is low. Soil is confirmed as the principal infection route and although the disease is self-eliminating, when infected crops are grown in PMTV-free soil, infected tubers may carry the infection to new sites. It can be concluded that PMTV is likely to be a manageable disease in the context of potato cultivation in Scotland, if an integrated control approach is adopted incorporating seed health checks, cultivar and site selection. Furthermore, the risk that PMTV poses to importers of Scottish seed potatoes, particularly those in warmer parts of the World is relatively low, as demonstrated by the marked temperature effect on PMTV infection and symptom development.

Although the body of work presented here contributes to the further understanding of the epidemiology of PMTV, a number of opportunities for further work in this area remain. Specifically, results presented here are based on a limited number of cultivars and further work to investigate the susceptibility and symptom expression of a broader range of cultivars is now required. Further refinement of the soil bait test is also required in order to quantify risk and to determine pathogen distribution at the field level. Further investigation would also be invaluable in determining why some populations of *Spongospora subterranea* serve as vectors for PMTV and others do not. Finally other environmental factors such as water availability, soil type, etc. should be investigated to determine their role in the infection process.

Appendix 1

Protocols for the preparation of buffers required for DAS-ELISA.

Appendix 1

DAS-ELISA buffers

Coating buffer

Reagent	Amount (g)
$Na_2CO_3.10H_2O$	4.29
$NaHCO_3$	2.93
ddH_2O	1000 ml

Stored at 4°C and used within two days of preparation.

PBSTween (PBST) buffers

Used as standard PBST buffer or concentrated PBST (10X).

Reagent	Amount (g)
NaCl	80
KH_2HPO_4	2
Na_2HPO_4	29
KCl	2
ddH_2O	10000 ml

0.05% Tween-20 (aq., v/v) was added after all the above were fully dissolved. All reagents were increased 10 fold for concentrated PBST.

Extraction buffer for leaf tissue

Reagent	Amount (g)
Polyvinylpyrrolidone (PVP)	20
Standard PBST buffer	1000 ml

Stored at 4°C and used within 2 days of preparation.

Note: Polyvinylpyrrolidone (PVP) must be weighed in a fume cupboard.

Tuber extraction buffer

Reagent	Amount (g)
Chicken egg albumin	10
Polyvinylpyrrolidone (PVP)	20
Standard PBST buffer	1000 ml

Stored at 4°C and used within 2 days of preparation.

Note: Polyvinylpyrrolidone (PVP) must be weighed in a fume cupboard.

Conjugate buffer

Reagent	Amount (g)
Bovine serum albumin (BSA)	0.2
Polyvinylpyrrolidone (PVP)	2
Standard PBST buffer	100 ml

Prepared fresh daily

Substrate buffer

Reagent	Amount (ml)
Diethanolamine (DEA)	97

DEA was first added to 800ml ddH_2O. The pH was adjusted to 9.8 using concentrated HCl (11.8 M). The volume was then made up to 1 litre using ddH_2O.

Stored at 4°C.

Note: Diethanolamine (DEA) must be measured in a fume cupboard.

Appendix 2

Results of a survey to determine the distribution of PMTV in the four major seed growing regions of Scotland. Tubers of four commonly grown cultivars were tested for PMTV and assessed for the presence of spraing symptoms

A) Hermes

	County	% PMTV*	% Spraing†
	Aberdeen	0.00	0.00
	Aberdeen	0.00	0.00
	Aberdeen	0.00	0.00
	Angus	0.00	0.00
	Angus	2.67	0.00
	Angus	3.33	0.67
	Angus	0.67	0.67
	Angus	0.00	0.00
	Angus	4.00	0.00
	Angus	0.00	0.00
	Angus	2.00	0.67
	Banff	0.00	0.00
	Banff	0.00	0.00
	Berwick	0.00	0.00
	Berwick	0.00	0.00
	Berwick	0.00	0.00
	Berwick	0.00	0.00
	Berwick	0.00	0.00
	Fife	1.33	0.00
	Kincardine	0.00	0.00
	Moray	0.00	1.33
	Moray	4.00	0.00
	Moray	2.00	0.00
	Nairn	0.00	0.00
	Perth	0.67	0.00
	Ross	0.00	0.00
	Ross	0.00	0.00
	Ross	0.00	0.00
	Ross	1.33	0.00
	Ross	0.00	0.00
	Ross	0.00	0.00
	Ross	0.00	0.00
	Roxburgh	0.00	0.00
	Roxburgh	0.00	0.00
	Roxburgh	0.67	0.00
Total	**35**	**11 (31%)**	**4 (11%)**

*- PMTV infection was determined by DAS-ELISA as described in Section 2.2.4
†- The presence of spraing was assessed using the method described in Section 2.2.3

B) Maris Piper

	County	% PMTV	% Spraing
	Aberdeen	1.33	0.00
	Aberdeen	0.00	0.00
	Aberdeen	0.00	0.00
	Angus	0.67	0.00
	Angus	0.00	0.00
	Angus	2.00	0.00
	Angus	22.67	0.00
	Angus	0.00	0.00
	Angus	30.00	0.00
	Angus	0.00	0.00
	Angus	0.00	0.00
	Angus	0.00	0.00
	Banff	0.00	0.00
	Banff	0.00	0.00
	Berwick	2.67	0.00
	Berwick	0.00	0.00
	Caithness	0.00	0.00
	Caithness	0.00	0.00
	Dumfries	0.00	0.00
	East Lothian	0.00	0.00
	East Lothian	0.67	0.00
	East Lothian	2.67	0.00
	Inverness	0.00	0.00
	Kincardine	0.00	0.00
	Kincardine	0.67	0.00
	Kincardine	6.67	0.00
	Moray	0.00	0.00
	Nairn	0.00	10.67
	Nairn	0.00	0.00
	Perth	0.67	18.00
	Ross	0.00	0.00
	Ross	0.00	0.00
	Ross	0.00	4.67
	Ross	0.00	0.00
	Ross	58.67	0.00
	Ross	0.00	1.33
	Roxburgh	0.00	0.00
	Roxburgh	0.00	0.00
	Roxburgh	0.00	0.00
	Sutherland	22.67	0.67
Total	**40**	**13 (33%)**	**5 (13%)**

C) Nicola

	County	% PMTV	% Spraing
	Aberdeen	0.67	0.00
	Aberdeen	0.67	0.00
	Aberdeen	0.00	0.00
	Aberdeen	0.00	0.00
	Angus	2.67	0.00
	Angus	8.00	1.33
	Angus	16.67	0.00
	Angus	0.00	0.00
	Angus	7.33	0.00
	Angus	0.67	0.00
	Angus	51.94	25.58
	Angus	82.00	9.33
	Caithness	0.00	0.00
	Inverness	0.00	0.67
	Kincardine	0.67	0.00
	Kincardine	26.00	0.00
	Kincardine	17.33	3.33
	Kincardine	3.33	0.00
	Kincardine	0.00	0.00
	Moray	1.33	2.67
	Nairn	2.00	0.00
	Perth	0.00	0.67
	Perth	0.00	0.00
	Ross	0.00	0.00
	Ross	0.00	0.00
	Ross	12.67	0.67
	Roxburgh	0.00	2.00
Total	**27**	**16 (60%)**	**9 (33%)**

D) Saturna

	County	% PMTV	% Spraing
	Aberdeen	0.00	0.00
	Aberdeen	0.67	1.33
	Aberdeen	0.00	0.00
	Aberdeen	0.00	0.00
	Aberdeen	0.00	0.00
	Angus	0.00	0.00
	Angus	4.00	0.67
	Angus	0.00	0.00
	Angus	18.67	10.67
	Angus	2.00	2.00
	Angus	0.00	0.00
	Angus	0.00	0.00
	Banff	0.00	0.00
	Berwick	0.00	0.00
	Fife	1.33	0.00
	Kincardine	0.00	0.00
	Moray	1.33	0.00
	Moray	0.00	0.00
	Moray	0.00	0.00
	Perth	0.00	0.00
	Perth	0.00	0.00
	Perth	14.67	4.00
	Ross	0.00	0.00
	Ross	5.33	0.00
	Roxburgh	0.00	0.00
	Roxburgh	0.00	5.33
Total	**26**	**8 (31%)**	**6 (23%)**

Grand Total	**128**	**48 (38%)**	**24 (19%)**

Bibliography

Adams, M.J., J.F. Antoniw and J.G.L Mullins (2001). Plant virus transmission by plasmodiophorid fungi is associated with distinctive transmembrane regions of virus-encoded proteins. *Archives of Virology* **146:** 1139-1153

Adams, M.J. (1991). Transmission of plant viruses by fungi. *Annals of Applied Biology* **11:** 479-492

Adams, M.J., P.J. Read, D.H. Lapwood, G.R. Cayley and G.A. Hide (1986). The effect of irrigation on powdery scab and other tuber diseases of potatoes. *Annals of Applied Biology* **110:** 287-294

Agrios, G.N. (2005). Plant pathology. Fifth Edition. Elsevier Academic Press 2005.

Ahmad, I., S. Iftikhar, U. Merz and M.H. Soomro (1996).First report of *Spongospora subterranea* on potato in Pakistan. *Plant Disease* **80:** 959

Andersen, B. A. B., M. Nicolaisen and S. L Nielsen (2002). Alternative hosts for potato mop-top virus, genus Pomovirus and its vector *Spongospora subterranea* f. sp. *subterranea*. *Potato Research* **45**: 37-43

Andret-Link, P. and M. Fuchs (2005). Transmission specificity of plant viruses by vectors. *Journal of Plant Pathology* **87:** 153-165

Andrivon, D. (1996). The origin of *Phytophthora infestans* populations present in Europe in the 1840s: a critical review of historical and scientific evidence. *Plant Pathology* **45**: 1027-1035

Anon (1976). Manual of Plant Growth Stages and Disease Assessment Keys. MAFF Publications, Pinner, Middlesex

Arif, M. and B. Reavy (2000). In vitro transcription and translation of potato mop-top Pomovirus RNA 3: Coat protein and read-through cistron. *Pakistan Journal of Biological Sciences* **3** (2): 303-305

Arif, M. and L. Torrance (1996). Detection of potato mop-top virus in potato tubers. *Proceedings Crop Protection in Northern Britain* pp. 325-330

Arif, M., L. Torrance and B. Reavy (1994). Improved efficiency of detection of potato mop-top furovirus in potato tubers and in the roots and leaves of soil-bait plants. *Potato Research* **37:** 373-381

Arif, M., L. Torrance and B. Reavy (1995). Acquisition and transmission of potato mop-top furovirus by a culture of *Spongospora subterranea* derived from a single cystosorus. *Annals of Applied Biology* **126:** 493-503

Arnold, D.L., A. Flegman and J.M. Clarkson (1995). Somaclonal variation in watercress for resistance to crook root disease. *Plant Cell Reports* **14:** 241-244

Barker, H and J. A. T. Woodford (1987). Unusually mild symptoms of potato leafroll virus in the progeny of late-infected mother plants. *Potato Research* **30** 345-348

Bell, K.S., J. Roberts, S. Verrall, D.W. Cullen, N.A. Williams, J.G. Harrison, I.K. Toth, D.E.L. Cooke, J.M. Duncan and J.R. Claxton (1999). Detection and quantification of *Spongospora subterranea* f. sp. *subterranea* in soils and tubers using specific PCR primers. *European Journal of Plant Pathology* **105:** 905-915

Bjornstad. A., (1969). The spread of potato mop-top virus (PMTV).with seed potato). *Jord og Avling* 2: 2-4

Boonham, N., K. Walsh, R.A. Mumford and I. Barker (2000). Use of multiplex real-time PCR (TaqMan).for the detection of potato viruses. *EPPO Bulletin* **30:** 426-430

Bouzoubaa, S., V. Ziegler, D. Beck, H. Guilley, K. Richards and G. Jonard (1986). Nucleotide sequence of beet necrotic yellow vein virus RNA 2. *Journal of General Virology* **67:** 1689-1700

Brierley, J. L., J. A. Stewart and A. K. Lees (2009). Quantifying potato pathogen DNA in soil. *Applied soil ecology* **41:** 234-238

British Potato Council (2007). Managing the risk of powdery scab information leaflet

Brown, C.R. (1993). Origin and history of the potato. *American Journal of Potato Research* **70**: 363-373

Brunt, A., K. Crabtree and A. Gibbs [*eds*.] Viruses of Tropical Plants – Descriptions and lists from the VIDE database. CAB International in association with the Australian centre for International Agricultural Research (1990). 707pp

Bulman, S.R. and J.W. Marshall (1998). Detection of *Spongospora subterranea* in potato tuber lesions using the polymerase chain reaction (PCR). *Plant Pathology* **47:** 759-766

Burton, W.G. (1989). The Potato. Longman Group UK Ltd 1989. p. 596

CAB International/EPPO (2002). Potato mop-top virus. Distribution maps of Plant Diseases. Map No. 874. Edition 1.

Calvert, E.L. (1968). The reaction of potato varieties to Potato mop-top virus. *Record of Agricultural Research* **17:** 31-40

Calvert, E.L. and B.D. Harrison (1966). Potato mop-top, a soil borne virus. *Plant Pathology* **15:** 31-40

Campbell, R.N. and P.R. Fry (1966). The nature of association between Olpidium brassicae and Lettuce big vein and Tobacco necrosis viruses. *Virology* **29:** 222-233

Carling, D.E. (1996). First report of powdery scab in Alaska. *Plant Disease* **80:** 1208

Carnegie, S. F, T. Davey and G. S. Saddler (2009). Effect of temperature on thetransmission of *Potato mop-top virus* from seed tuber and by vector, *Spongospora subterranea*. *Plant Pathology*, in press. Doi: 10.1111/j.1365-3059.2009.02187.x.

Carnegie, S. F, G. S. Saddler, T. Davey, J. Peters, G. Budge (2009). Testing potato varieties for susceptibility to potato mop-top virus. Project report to Potato Council Ltd, 23pp.

Čeřovská, N., T. Pečenková, M. Filigarová and P. Dědič (2007). Sequence analysis of the Czech potato mop-top virus (PMTV).Isolate Korneta-Nemilkov. *Folia Microbiologica* **52**: 61-64

Chapman, J. (2000). The impact of the potato. *History Magazine*. December/January Edition 2000.

Clay, C. M. and J. A. Walsh (1997). *Spongospora subterranea* f sp. *nasturtii*, ultrastructure of the plasmodial-host interface, food vacuoles, flagellar apparatus and exit pores. *Mycological Research* **101**: 737-744

Cohen, J. (1960). A coefficient of agreement for nominal scales. *Education and Psychological Measurement* **20**: 37-46

Connell, K.H. (2002). The potato in Ireland. In: Philpin, C.H.E. [*ed*.] Nationalism and popular protest in Ireland. Cambridge University press 2002. p480

Cooper, J.I. and B.D. Harrison (1973).The role of weed hosts and the distribution and activity of vector nematodes in the ecology of tobacco rattle virus. *Annals of Applied Biology* **73:** 53-66

Cooper, J.I. and B.D. Harrison (1973). Distribution of Potato mop-top virus in Scotland in relation to soil and climate. *Plant Pathology* **22:** 73-78

Cooper, J.I, R.A.C. Jones and B.D. Harrison (1976). Field and glasshouse experiments on the control of potato mop top virus. *Annals of Applied Biology* **83:** 215-230

Cowan, G.H., Torrance, L. and B. Reavy, B (1997).Detection of potato mop-top virus capsid readthrough protein in virus particles. *Journal of General Virology* **78:** 1779-1783

Dale, F. and Neilson, R. (2006). Free Living Nematodes and Spraing. Research Review. British Potato Council. Ref: R276

DARDNI (Department of Agriculture and Rural Development Northern Ireland).(2007).Northern Ireland Register of Pre-Basic *and* Basic Seed Potato Crops 2007. Northern Ireland Government 2007. Available online at: http://www.dardni.gov.uk/seed-register-2007

De Bary, A. (1884). *Morphologie und Biologie der Pilze, Mycetozoen und Bacterien*. W. Engelmann, Leipzig

De Boer, R.F. (2000). Research into the biology and control of powdery scab of potatoes in Australia. In: Merz, U. and A.K. Lees [*eds.*] Proceedings of the first European Powdery Scab Workshop, 2000. Aberdeen, Scotland, July 20-22

De Boer R.F., P.A. Taylor, S.P. Flett and P.R. Merriman (1985). Effects of soil temperature, moisture and timing of irrigation on powdery scab of potatoes. In: C.A. Parker, K.J. Moore, P.T.W. Wong, A.D. Rovira and J.F. Kollmorgen [*eds.*] Ecology and Management of Soil-Borne Plant Diseases. 197-198. American Phytopathological Society, St Paul, Minnesota, USA.

De Haan, E.G. and G.W. van den Bovenkamp (2005). Improved diagnosis of powdery scab (*Spongospora subterranea* f. sp. *subterranea*).symptoms on potato tubers (Solanum tuberosum L.). *Potato Research* **48:** 1-14

De Hoop, M.B., J. T. J. Verhoeven and J.W. Roenhorst (2008). Phytosanitary measures in the European Union: a call for more dynamic risk management allowing more focus on real

pest risks. Case study: Potato sindle tuber viroid (PSTVd).on ornamental Solanaceae in Europe. *European and Mediterranean Plant Protection organisation Bulletin* **38**: 510-515

Dessens, J.T. and M. Meyer (1996). Identification of structural similarities between putative transmission proteins of *Polymyxa* and *Spongospora* transmitted bymoviruses and furoviruses. *Virus Genes* **12:** 95-99

Down, G. J., L. J. Grenville and J. M Clarkson (2002). Phylogenetic analysis of *Spongospora* and implications for the taxonomic status of the plasmodiophorids. *Mycological Research* **106** (9): 1060-1065

Draper, M.A., G.A. Secor and N.C. Gudmestad (1997). First report of potato powdery scab, caused by *Spongospora subterranea* f.sp. *subterranea* in North Dakota. Plant Disease **81:** 693-697

European Union (2002).Council Directive 2002/56/EC of 13 June 2002 on the marketing of seed potatoes.

European Union (1993).Commission Directive 93/17/EEC of 30 March 1993 determining Community grades of basic seed potatoes, together with the conditions and designations applicable to such grades

Falloon, R. E., A. R. Wallace, M. Braithwaite, R. A. Genet, H. M. Nott, J. D. Fletcher, W. F. Braam (1996). Assessment of seed tuber, in-furrow, and foliar chemical treatments for control of powdery scab (*Spongospora subterranea* f. sp. *subterranea*).of potato. *New Zeland Journal of Crop Horticultural Science* **24**: 341-353

FAO (2008a). Potatoes nutrition and diet. FAO Fact sheet. FAO (Food and Agriculture Organisation of the United Nations).

FAO (2008b). The global potato economy. FAO Fact sheet. FAO (Food and Agriculture Organisation of the United Nations).

FERA (Food and Environment Research Agency).(2008).Provisional summary of applications to the Seed Potato Classification Scheme England and Wales 2008. Available online at: http://www.fera.defra.gov.uk/plants/plantHealth/documents/spcs08.pdf

Foxe, M.J. (1980). An investigation of the distribution of potato mop-top virus in County Donegal. *Journal of Life Sciences Royal Dublin Society* **1:** 149-155

Francki, C.M. Fauquet, D.L.Knudson, F.Brown (1991). Fifth Report of the International Committee on Taxonomy of Viruses. Archives of Virology, Springer Verlag, Wien New York

Germundsson, A., M. Sandgren, H. Barker, E.I. Savenkov and J.P.T. Valkonen (2002). Initial infection of roots and leaves reveals different resistance phenotypes associated with coat-protein gene-mediated resistance to potato Mop-Top Virus. *Journal of General Virology* **83:** 1201-1209

Germundsson, A., M. Sandgren, E. Savenkov, J. Valkonen (2000). Spraing in potato - a complicated problem with several causes. *Vaxtskyddsnotiser* **64**: (3-4), 51-58

Glave, L.M. (2001). The conquest of the highland. In Graves, C. [*ed*.], The potato, treasure of the Andes; From agriculture to culture. International Potato Centre (CIP), Peru 2001.

Goulden, M.G., G.P. Lomonossoff, K.R. Wood and J.W. Davies (1991). A model for the generation of tobacco rattle virus (TRV).anomalous isolates: pea early browning virus RNA-2 acquires TRV sequences from both RNA-1 and RNA-2. *Journal of General Virology* **72:** 1751-1754

Harding, S.G. (1993). The "racial" economy of science; toward a democratic future. Indiana University Press. p. 526

Hariri, D and M. Meyer (2007). A new furovirus infecting barley in France closely related to the Japanese soil-borne wheat mosaic virus. *European Journal of Plant Pathology* **118**: 1-10

Harrison, B. D. (1974). Potato mop-top virus. *CMI/AAB Description of Plant Viruses* No. 138

Harrison, B.D. and R.A.C. Jones (1971a). Factors affecting the development of spraing in potato tubers infected with potato mop-top virus. *Annals of Applied Biology* **68**: 281-289

Harrison, B.D. and R.A.C. Jones (1971b). Effects of light and temperature on symptom development and virus content of tobacco leaves inoculated with potato mop-top virus. *Annals of Applied Biology* **67**: 377-387

Harrison, B.D. and R.A.C. Jones (1970). Host range and properties of potato mop-top virus. *Annals of Applied Biology* **65:** 393-402

Harrison, B.D. and B. Reavy (2002). Potato mop-top virus. Descriptions of Plant Viruses AAB No. 389. Accessed online: www.dpvweb.net/dpv/showadpv.php?dpvno=389

Harrison, J.G., E.A. Rees, H. Barker and R. Lowe (1993). Detection of spore balls of *Spongospora subterranea* on potato tubers by enzyme-linked immunosorbent assay. *Plant Pathology* **42:** 181-186

Harrison, J.G., R.J. Searle and N.A. Williams (1997). Powdery scab disease of potato – a review. *Plant Pathology* **46:** 1-25

Haupt, S. G.H. Cowan, A. Ziegler, A.G. Roberts, K.J. Oparka and L. Torrance (2005). Two plant-viral movement proteins traffic in the endocytic recycling pathway. *The Plant Cell* **17:** 164-181

Helias V. and D. Bourdin (2000). Evaluation and adaption of diagnostic methods for Potato mop-top virus (PMTV). In: Merz, U. and A.K. Lees [*eds.*] Proceedings of the first European Powdery Scab Workshop, 2000. Aberdeen, Scotland, July 20-22

Hijmans, R.J. and D.M. Spooner (2001). Geographic distribution of wild potato species. *American Journal of Botany* **88**: 2101-2112

Hims, M.J. and T.F. Preece (1975). *Spongospora subterranea*. CMI Descriptions of Pathogenic Fungi and Bacteria, no. 477.

Hinostroza, A.-M. and E.R. French (1972). Potato mop-top virus in cork diseased Peruvian potatoes. *American Potato Journal* **49:** 234-239

HMSO (1981). Powdery scab of potatoes. Ministry of Agriculture, Fisheries and Food, Leaflet No. 5

Holeva, R. M. S. Phillips, R. Neilson, D. J. F. Brown, V. Young, K. Boutsika and V. C. Blok (2006). Real-time PCR detection and quantification of vector trichodorid nematodes and *Tobacco rattle virus*. *Molecular and Cellular Probes* **20** (3-4): 203-211

Hosaka, K. and R.E. Hanneman, Jr. (1988). The origin of the cultivated tetraploid potato based on chloroplast DNA. *Theoretical and Applied Genetics* **76**: 172-176

Hougas, R.W. (1956). Foreign potatoes, their introduction and importance. *American Journal of Potato Research* **33**: 190-198

Huaman, Z and D.M. Spooner (2002). Reclassification of landrace populations of cultivated potatoes (*Solanum* Sect. *Petota*). *American Journal of Botany* **89**: 947-965

ICTV (International Committee on Taxonomy of Viruses) (2002). Genus Pomovirus. Index classification, online: www.ncbi.nlm.nih.gov/ICTVdb/Ictv/fs_pomov.htm

Imoto, M., M. Iwaki, H. Tochihara, K. Nakamura and K. Hanada (1986). Occurrence of potato mop-top virus in Japan and some of its properties. *Annals of the Phytopathological Society of Japan* **52:** 752-757

Jeffries, C. J. (1998). Potato. In FAO/IPGRI *Technical guidelines for the safe movement of Germplasm*, no. 19. Rome, Italy: Food and Agricultural Organisation of the United Nations/International Plant Genetic Resources Institute

Jeffries, R. A. and H. M. Lawson (1991). A key for the stages of development of potato (*Solanum tuberosum*). *Annals of applied Biology* **119**: 389-399

Ji, Y.B., S.Y. Gao, C.F. Ji, and X. Zou (2008). Induction of apoptosis in $HepG_2$ cells by solanine and Bcl-2 protein. *Journal of Ethnopharmacology* **115**: 194-202

Jones, R.A.C (2007). Hypothesis to explain how viruses induce production of local lesions in plant tissues.Bulletins of the F. G. W. Jones Family Historical Series. Bulletin No. 2

Jones, R.A.C. (1988a). Potato mop-top virus. In: Hooker, W.J. [*ed*.] Compendium of potato diseases. APS press, St Paul, Minnesota. pp79-80

Jones, R.A.C. (1988b). Epidemiology and control of Potato mop-top virus. Developments in Applied Biology II. In: Cooper, J.I. and M.J.C. Asher [eds.] Viruses with fungal vectors. Proceedings of a conference at the University of St Andrews, August 25-27, 1987. Wellesboure, UK.

Jones, R.A.C (1981). In: *Pests, Pathogens and Vegetation: the role of weeds and wild plants in the ecology of crop pests and diseases*; ed. J.M. Thresh. Pitman, London.

Jones, R.A.C (1975). Presence of mop-top virus in Bolivia. *Plant Distribution Reports* **59:** 193

Jones, R.A.C. and B.D. Harrison (1972). Ecological studies on potato mop-top virus in Scotland. *Annals of Applied Biology* **71:** 47-57

Jones, R.A.C. and B.D. Harrison (1969). The behaviour of potato mop-top virus in soil, and evidence for its transmission by *Spongospora subterranea* (Wallr.).Lagerh. *Annals of Applied Biology* **63:** 1-17

Kallender, H. K.W. Buck and A.A. Brunt (1990). Association of three RNA molecules with potato mop-top virus. *Netherlands Journal of Plant Pathology* **96:** 47-50

Karling, J.S. (1968). The Plasmodiophorales, 2nd Edition. Hafner Publishing, New York.

Kashiwazaki, S., K.P. Scott, B. Reavy and B.D. Harrison (1995).Sequence analysis and gene organisation of potato mop-top virus RNA-3: further evidence of hererogeneity in the genome organisation of furoviruses. *Virology* **206:** 701-706

Kaur, S. and K.G. Mukerji (2004). Potato diseases and their management. In: Mukerji, K.G. [*ed*.], Disease Management of fruit and vegetables Volume 1. Kluwer Academic Publishers 2004.

Kim, J.S., K.Y. Ryu, J.T. Kim, Y.G. Lee, J.U. Cheon (2003). Occurrence of potato powdery scab caused by *Spongospora subterranea* in Korea. *The Plant pathology Journal* **19:** 284-287

Kinealy, C. (1997). A death-dealing famine; The great hunger in Ireland. Pluto Press. p 200.

Kirk, H. G. (2008). Mop-top virus, relationship to its vector. *American Journal of Potato research* **85**: 261-265

Knapp, S., L. Bohs, M. Nee, and D.M. Spooner (2004). Solanaceae – a model for linking genomics with biodiversity. *Comparative and Functional Genomes* **5**: 285-291

Koenig, R., C.W. Pleij, C. Beier and U. Commandeur (1998). Genome properties of beet virus Q, a new furo-like virus from sugarbeet, determined from unpurified virus. *Journal of General Virology* **79:** 2027-2036

Kole, A.P. and A.J. Gielenk (1963). The significance of the zoosporangial stage in the life cycle of the plasmodiphorales. *Netherlands Journal of Plant Pathology* **69:** 258-262

Korpan, Y.I., E.A. Nazarenko, I.V. Skryshevskaya, C. Martelet, N. Jaffrezic-Renault and A.V. El'skaya (2004). Potato glycoalkaloids: true safety or false sense of security? *Trends in Biotechnology* **22**: 147-151

Kurppa, A. (1989a). Reaction of potato cultivars to primary and secondary infection by potato mop-top furovirus and strategies for virus detection. *EPPO Bulletin* **19:** 593-598

Kurppa, A. (1989b). The distribution and incidence of potato mop-top virus in Finland as determined in 1987 and on the variation of disease symptoms in infected potatoes. *Annales Agriculturae Fenniae* **28**: 285-295

Lambert, D.H., L. Levy, V.A. Mavrodieva, S.B. Johnson, M.J. Babcock and M.E. Vayda (2003). First Report of Potato mop-top virus on Potato from the United States. *Plant Distribution* **87**: 872

Langkilde, S., M. Schroder, D. Stewart, M. Otto, S. Connor, H. Davies and M. Poulsen (2008). Acute toxicity of high doses of the glycoalkaloids, α-Solanine and α-Chaconine, in the Syrian Golden Hamster. *Journal of Agricultural and Food Chemistry* **56**: 8753-8760

Latvala-Kilby, S., J. M. Aura, N. Pupola, A. Hannukkala and J. P. T. Valkonen (2009). Detection of *Potato mop-top virus* in potato tubers and sprouts: combinations of RNA2 and RNA3 variants and incidence of symptomless infections. *Phytopathology* **99** (5): 519-531

Laufer, B., and W.B. Martin (1938). The American Plant Migration Part 1: The potato. Chicago Field Museum Press 1938.

Lee, L, E.B. Telford, J.S. batten, K.-B.G. Scholthof and C.M. Rush (2001). Complete nucleotide sequence and genome organisation of Beet soil-borne mosaic virus, a proposed member of the genus Benyvirus. *Archives of Virology* **146:** 2443-2453

Legorburu, F.J., D.J. Robinson and L. Torrance (1996). Features on the surface of the tobacco rattle tobravirus particle that are antigenic and sensitive to proteolytic digestion. *Journal of General Virology* **77:** 855-859

Lukhovitskaya, N.I., N.E. Yelina, A.A. Zamyatnin Jr, M.V. Schepetilnikov, A.G. Solovyev, M. Sandgren, S. Y. Morozov, J.P.T. Valkonen and E.I. Savenkov (2005).

Expression, localisation and effects on virulence of the cysteine-rich 8 kDa protein of Potato mop-top virus. *Journal of General Virology* **86:** 2879-2889

Mayo, M. A., L. Torrance, G. Cowan, C. A. Jolly, S. M. Macintosh, R. Orrega, C. Barrera, L. F. Salazar (1996). Conservation of coat protein sequence among isolates of potato mop-top virus from Scotland and Peru. *Archives of Virology* **141**: 1115-1121

McGeachy, K.D. and H. Barker (2000). Potato mop-top virus RNA can move long distance in the absence of coat protein: Evidence from resistant, transgenic plants. *Molecular Plant-Microbe Interactions* **13:** 125-128

Merz, U. (2008). Powdery scab of potato – occurrence, life cycle and epidemiology. *American Journal of Potato Research* **85:** 241-246

Merz, U. (2000). Powdery scab research in Switzerland. In: Merz, U. and A.K. Lees [*eds.*] Proceedings of the first European Powdery Scab Workshop, 2000. Aberdeen, Scotland, July 20-22

Merz, U. (1992). Observations on swimming pattern and morphology of secondary zoospores of *Spongospora subterranea*. *Plant Pathology* **41**: 490-494

Merz, U., J.A. Walsh, K. Bouchek-Mechiche, Th. Oberhansli and W. Bitterlin (2005). Improved immunological detection of *Spongospora subterranea*. *European Journal of Plant of Plant Pathology* **111:** 371-379

Merz, U. and J.A. Walsh (1997). Serological detection of *Spongospora subterranea* f. sp. subterranean in soil. In: Dehne, H.W, G. Adam, M. Diekmann, J. Frahm, A. Mauler-Machnik and T.J. van Halteren Peters [*eds.*] *Diagnosis and Identification of Plant Pathogens*. Proceedings of the 4th International Symposium of the European Foundation for Plant Pathology. Kluwer Academic Publishers, Netherlands 1997.

Mohammadi, A., A. Tabatabaeefar, S. Rafiee, A. Keyhani (2008). Energy use and economical analysis of potato production in Iran a case study: Aradabil Provence. *Energy Conversion and management* **49:** (12).3566-3570

Mølgaard, J. P. and S. L. Nielsen (1996). Influence of post harvest temperature treatments, storage period and harvest on development of spraing caused by tobacco rattle virus and potato mop-top virus. *Potato Research* **39**: 571-579

Montero-Austua, M., V. Vasquez, W. W. Turechek, U. Merz, and C. Rivera (2002).Occurrence of potato powdery scab caused by *Spongospora subterranea* f.sp. subterranea in Costa Rica. *Plant disease* 86: 1273

Mulder, A. and L.J. Turkensteen (2005). Introduction. In: Mulder, A. and L.J. Turkensteen [*eds*.] Potato diseases. Diseases, pests and defects p.11-12. Aardappelwereld B.V. and NIVAP 2005.

Müller, J.L. (1998). Love potions and the ointment of witches: Historical aspects of the Nightshade alkaloids. *Journal of toxicology. Clinical toxicology* **36**: 617-627

Mumford, R. A., K. Walsh, I. Barker and N. Boonham (2000). Detection of *potato mop top virus* and *tobacco rattle virus* using multiplex real-time fluorescent reverse-transcriptase polymerase chain reaction assay. *Phytopathology* **90:** 448-453.

Nakayama, T., M. Horita and T. Shimanuki (2007). *Spongospora subterranea* soil contamination and its relationship to severity of powdery scab on potatoes. *Journal of General Plant Pathology* **73:** 239-234

Nielsen, S.L and J.P. Molgaard (1997). Incidence, appearance and development of potato mop-top furovirus-induced spraing in potato cultivars and the influence on yield, distribution in Denmark and detection of the virus in tubers by ELISA. *Potato Research* **40:** 101-110

Nielsen, S. L. and M. Nicolaisen (2003). Identification of two nucleotide sequence sub-groups within potato mop-top virus. *Archives of Virology* **148**: 381-388

Nielsen, S. L. and M. Nicolaisen (2000). National potato production and the powdery scab situation in Denmark. In: Merz, U. and A.K. Lees [*eds.*] Proceedings of the first European Powdery Scab Workshop, 2000. Aberdeen, Scotland, July 20-22

Nielsen, S. L., K. R. Hansen and F. Trosburg (2003). Potato mop-top virus in Denmark – status and perspectives. In: *20. Danske Planteværnskonference*, DJF rapport - Markbrug 89. *2003*. p. 125-136

Novak, J.B., V. Rasocha and J. Lancova (1983). Novak, J.B. Rasocha, V. & Lanzova, J. 1983. Demonstration of potato mop-top virus in the Czechoslovak Socialist Republik. *Sbor. UVTIZ Ochr. Rostl* **19:** 161-167.

Ochoa, C. (2001). Universal Gift. In Graves, C. [*ed.*], The potato, treasure of the Andes; From agriculture to culture. International Potato Centre (CIP), Peru 2001.

OECD (2008). Safety assessment of transgenic organisms. OECD Consensus Documents Volumes 1 and 2. OECD (Organisation for Economic Cooperation and Development).2008.

Pečenková, T., T. Moravec, M. Filigarová, P. Rosecká and N. Čeřovská (2004). Extended sequence analysis of three Danish potato mop-top virus (PMTV).isolates. *Virus Genes* **29**: 249-255

Porta-Puglia, A. and Mifsud, D. (2006). First record of powdery scab caused by *Spongospora subterranea subsp. subterranea* on potato in Malta. *Journal of Plant Pathology 88:* 225-229

Potato Council Ltd. (2008). Potatoes: The facts. A definitive guide to the nutritional content of raw and cooked potatoes. Potato Council Ltd 2008.

Qu, Xinshun and B.J. Christ (2006). The host range of *Spongospora subterranea* f. sp. *subterranea* in the United States. *American Journal of Potato Research* **83:** 343-347

Radcliffe, E. B. and A. Lagnaoui (2007). Insect pests in potato. In: Vreugdenhil, V. [*ed*.] Potato biology and biotechnology. Elsevier 2007. p541.

Reader, J, (2008). Propitious esculent: The potato in world history. William Heinemann Ltd. February 2008.

Reavy, B. M. Sandgren, H. Barker, P. Heino and P. Oxefelt (1997). A coat protein transgene from a Scottish isolate of potato mop-top virus mediates strong resistance against Scandinavian isolates which have similar coat protein genes. *European Journal of Plant Pathology* **103:** 829-834

Reavy, B, M. Arif, G.H. Cowan and L. Torrance (1998). Association of sequences in the coat protein/readthrough domain of potato mop-top virus with transmission by *Spongospora subterranea*. *Journal of General Virology* **79:** 2343-2347

Rennie, W. J. (2001). Seed potato production in Scotland. Scottish Agricultural Science Agency Scientific Review 1997-2000 pp 40-45

Salaman, R.N., W.G. Burton and J.G. Hawkes (1985). The history and social influence of the potato. Cambridge University Press 1985 pp. 15

Salazar, L.F. and R.A.C. Jones (1975). Some studies on the distribution and incidence of Potato mop-top virus in Peru. *American Potato Journal* **52:** 143-150

Sandgren, M. (1995). Potato mop-top virus (PMTV): distribution in Sweden, development of symptoms during storage and cultivar trials in field and glasshouse. *Potato Research* **38:** 379-389

Sandgren, M., R. L. Plaisted, K. N. Watanabe, S. Olsson and J. P. T. Valkonen (2002). Evaluation of some North and South American potato breeding lines for resistance to Potato mop-top virus in Sweden. *American Journal of Patato Research* **79**: 205-210

Sandgren, M., E. Savenkov, and J.P.T. Valkonen (2001). The readthrough region of potato mop-top virus coat protein encoding RNA, the second largest RNA of PMTV genome, undergoes structural changes in naturally infected and experimentally inoculated plants. *Archives of Virology* **146:** 467-477

SASA (Science and Advice for Scottish Agriculture) (2007). Register of Pre-Basic and Basic seed potato crops 2007. Scottish Government 2007. Available online at: http://www.sasa.gov.uk/mediafiles/9450E42A_D093_17D6_C7FD18A201ECEDC1.pdf

Savenkov, E., M. Sandgren, J.P.T. Valkonen (1999). Complete sequence of RNA 1 and the presence of tRNA-like structures in all RNA's of Potato mop-top virus, genus Pomovirus. *Journal of General Virology* **80:** 2779-2784

Scott, K.P., S. Kashiwazaki, B. Reavy and B.D. Harrison (1994). The nucleotide sequence of potato mop-top virus RNA 2: A novel type of genome organisation for a furovirus. *Journal of General Virology* **75:** 3561-3568

Scottish Government (2000). The Seed Potatoes (Scotland).Regulations 2000. Scottish Statutory Instrument 2000 No. 20. Crown Copyright 2000

Scottish Government (2008). Scottish Government potato export data. Available at: www.scotland.gov.uk/Topics/Agriculture/plant/18273/PotatoExportData

Scottish Government (2009a). Economic Report on Scottish Agriculture, 2009 Edition. Scottish Government Rural and Environment Research and Analysis Directorate 2009.

Scottish Government (2009b) Summary of seed potato export volume by destination 2000-2009. Available at: http://www.scotland.gov.uk/Topics/farmingrural/Agriculture/plant/18273/potatoexpconds/data/seed/SummarySeedYearly Accessed on 8th December 2009.

Shirako, Y. & Wilson, T. M. A. (1993). Complete nucleotide sequence and organization of the bipartite RNA genome of soil-borne wheat mosaic virus. *Virology* **195:** 16-32.

Singh, D, N. Verma and A. Varma (2008). The fungal transmitted viruses. In: Varma, A. [*ed.*] Mycorrhiza: genetics and molecular biology, eco-function, biotechnology, eco-physiology, structure and systematics. p.485-503 Springer-Verlag, Berlin, Heidelberg 2008.

Sokmen, M. A., H. Barker and L. Torrance (1998). Factors affecting the detection of potato mop-top virus in potato tubers and improvement of test procedures for more reliable assays. *Annals of Applied Biology* **133:** 55-63

Stevenson, W.R., R. Loria, G.D. Franc, and D.P. Weingartner (2001). Compendium of potato diseases. APS Press, St Pauls, Minnesota. pp.144.

Taylor, C. E and W. M. Robertson (1970). Location of Tobacco Rattle Virus in the Nematode Vector, *Trichodorus pachydermus* Seinhorst. *Journal of General Virology* **6**: 179-182

Termorshuizen A. J (2007). Fungal and Fungus-like pathogens of potato. In: Vreugdenhil, V. [*ed*.] Potato biology and biotechnology. Elsevier 2007. p823.

Tenorio, J., Y. Franco, C. Chuquillanqui, R. A. Owens and L. F. Salazar (2006). Reaction of potato varieties to *Potato mop-top virus* infection in the Andes. *American Journal of Potato Research* **83:** 423-431

Todd, J. M. (1965). Soil-borne virus diseases of potato. *Report of The Scottish Plant Breeding Station*. 1965, pp: 209-235.

Torrance, L., G.H. Cowan, M.A. Sokmen and B. Reavy (1999). A naturally occurring deleted form of RNA 2 of Potato mop-top virus. *Journal of General Virology* **80:** 2211-2215

Torrance, L. and M.A. Mayo (1997). Proposed re-classification of furoviruses. *Archives of Virology* **142:** 435-439

Torrance, L., G. H Cowan, K. P. Scott, L. G. Pereira, I. M. Roberts, B. Reavy, and B.D. Harrison (1992). Detection and diagnosis of potato mop-top virus. Annual Report of Scottish Crop Research Institute for 1991, pp. 80-82

Towers, W., I. C. Grieve, G. Hudson, C. D. Campbell, A. Lilly, D. A. Davidson, J. R. Bacon, S. J. Langan and D. A. Hopkins (2006). Scotland's soil resource – current state and threats. Stirling University and Macaulay Institute.

True, R.H., J.M. Hogan, J. Augustin, S.J. Johnson, C. Teitzel, R.B. Toma and R.L. Shaw (1978). Mineral composition of freshly harvested potatoes. *American Journal of Potato Research* **55**: 511-519

Ugent, D., S. Pozorski, and T. Pozorski (1982). Archaeological potato tuber remains from the Casma Valley of Peru. *Economic Botany* **36**: 182-192

UK Meteorological Office (2009). UK Meteorological Office website: http://www.metoffice.gov.uk/climate/uk/averages/ukmapavge.html# (accessed 25th June 2009).

Valkonen, J.P.T. (2007). Viruses: Economical losses and Biotechnological potential. In: Vreugdenhil, V. [*ed*.] Potato biology and biotechnology. Elsevier 2007. p823.

Van de Graaf, P., A.K. Lees, S.J. Wale and J.M. Duncan (2005). Effect of soil inoculum level and environmental factors on potato powdery scab caused by *Spongospora subterranea*. *Plant Pathology* **54:** 22-28

Van de Graaf, P., A.K. Lees, D.W. Cullen and J.M. Cullen (2003). Detection and quantification of *Spongospora subterranea* in soil, water and plant tissue samples using real-time PCR. *European Journal of Plant Pathology* **109:** 589-597

Van de Graaf, P., A.K. Lees and J.M. Duncan (2002). Effect of soil inoculum level and environmental conditions on powdery scab and root galling in potato. Proceedings Crop Protection in Northern Britain 2002.

Van Hoof, H.A. and A. Rozendaal (1969). Het voorkomenvan 'potato mop-top virus' in Nederland. *Netherlands Journal of Plant Pathology* **75:** 275

Verchot-Lubicz, J. (2003). Soil-borne viruses: advances in virus movement, virus induced gene silencing, and engineered resistance. *Physiological and Molecular Plant Pathology* **62: 55-63**

Verchot-Lubicz, J., C.M. Rush, M. Payton and T. Colberg (2007). Beet necrotic yellow vein virus accumulates inside resting spores and zoosporangia of its vector Polymyxa betae BNYVV infects P.betae. *Virology Journal* **4:** 37

Wale (2003). Powdery scab fact file. Stuart Wale, SAC (Scottish Agricultural College).Aberdeen, July 2003.

Wale (2000). The powdery scab situation in Scotland. In: Merz, U. and A.K. Lees [*eds.*] Proceedings of the first European Powdery Scab Workshop, 2000. Aberdeen, Scotland, July 20-22

Wale, S., H.W. (Bud). Platt and N.Cattlin (2008). Diseases, pests and disorders of potatoes. A colourful handbook. Manson Publishing 2008.

Walsh, J.A., C.M. Clay and A. Miller (1988). A new virus disease of watercress in England. *European and Mediterranean Plant protection Organisation (EPPO).Bulletin.* **19:** 463-470

Ward, E. and M.J. Adams (1998). Analysis of ribosomal DNA sequences of Polymyxa species and related fungi and the development of genus- and species- specific PCR primers. *Mycological Research* **102:** 965-974

Ward, L.I., P.A. Beales, A.V. Barnes and C.R. Lane (2004). A real-time PCR assay based method for routine diagnosis of *Spongospora subterranea* on potato tubers. *Journal of Phytopathology* **152:** 633-638

Ward, L., R. Koenig, G. Budge, C. Garrido, C. McGrath, H. Stubbley and N. Boonham (2007). Occurrence of two different types of RNA-5-containing beet necrotic yellow vein virus in the UK. *Archives of Virology* **152:** 59-73

Wastie, R. L., P. D. S. Caligari and S. J. Wale (1988). Assessing the resistance of potatoes to powdery scab (*Spongospora subterranea* (Wallr. Lagerh.)). *Potato Research* **31**: 167-171

Xu, H., T.-L. DeHaan and S.H. De Boer (2004). Detection and confirmation of Potato mop-top virus in potatoes produced in the United States and Canada. *Plant Disease* **88:** 363-367

Zimmerer, K. (2002). Social and agroecological variability of seed production and the potential collaborative breeding of potatoes in the Andean countries. In: Cleveland, D.A. and D. Soleri. Farmers, scientists and plant breeding: Integrating knowledge and practice. CABI 2002. p338

Zimmerman-Gries, S. (1972). Effects of mop-top virus on potato in Israel. Actas Congress *Uniao Fitopathologia Mediterranea (Oliras)*.**3:** 375-383